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CONTINUATION OF THE STUDIES ON THE MECHANISM AND FUNCTION OF PERIPHERAL CHEMORECEPTORS

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Mieczysław Pokorski, Sylwia Elwich, Lidia Faff, Beata Kopczyńska, Małgorzata Musielak, Anna Nowakowska, Bogna M. Szereda-Przestaszewska

The studies performed concerned several aspects of the subcellular mechanisms of carotid body function. One of them had to do with the protein kinase C (PKC) in carotid body. The enzyme has been identified in the organ by an immunofluorescent technique. Experiments were performed on carotid bodies, dissected from anesthetized cats after preexposure to normoxia and acute hypoxia (10 min, 7% O_2 in N_2). The tissue was frozen in liquid nitrogen or fixed and processed according to the desired protocol. From the set of possible isoforms, alpha and delta PKC turned out to be most abundantly present in the carotid chemoreceptor cells. The preliminary studies with an immunogold technique at the electron microscopic level suggest that this isoform translocates towards the plasma membrane after exposure to the hypoxic stimulus. The results suggest that PKC has a part in the chemotransduction process. The exact role of PKC in this process remains to be established. The studies were performed in collaboration with the Center for Electron Microscopy, Leiden University (M. Pokorski, L. Faff).

Another aspect of carotid body mechanisms concerned the action of dopamine in the transduction process. A concept has been designed which presupposes that dopamine would be acylated by free fatty acids, notably the arachidonic acid, which would explain how dopamine could be stored in the dense-core vesicles of the chemoreceptor cells. Dopamine liberated from such a conjugate would exert its signalling effect. The existence of endogenous acyldopamine should be verified experimentally. The concept has been published in collaboration with the Institute of Biochemistry and Biophysics (M. Pokorski).

The mechanisms of inhibition of the hypoxic ventilatory response by phenylmethylsulphonyl fluoride (PMSF) were further elucidated. We assessed the concentration and distribution of the fluoride ion in chosen tissues of a cat. The results showed that degeneration of the carotid body parenchyma due to the action of PMSF could not be caused by the biotoxic effect of fluoride (M. Pokorski, M. Musielak).

Non-vagal respiratory effects of nicotine and serotonin, two substances evoking pulmonary chemoreflex were examined in cats. Increase in ventilation after nicotine challenge into the pulmonary circulation in vagotomized animals is due to excitation of carotid body chemoreceptors. Deafferentation of the latter precludes the hyperventilatory response. Expiratory apnoea and stimulation of the timing component of the breathing pattern evoked by serotonin occurs without contribution of the carotid chemoreflex and might depend on stimulation of serotonergic receptors on the nodose ganglia of the vagus nerve (B.M. Szereda-Przestaszewska, B. Kopczyńska).

EFFECT OF AMYLOID β (Aβ) UPON THE BIOCHEMICAL MECHANISM OF DNA INJURY AND NEURON(S) APOPTOSIS

Supported by the State Committee for Scientific Research: grant # 4.P05A.051.12

Research co-ordinator
Robert Strosznajder

The studies indicate that aggregated form of Aβ (1-40) inhibited Ca^{2+} regulated phosphatidylinositol and phosphatidylinositol-diphosphate degradation by synaptic plasma membranes (SPM) and cytosolic enzymes. Amyloid β (1-40) significantly decreased (muscarinic-cholinergic receptor

dependent, G protein regulated) phosphatidylinositol-diphosphate-phospholipase C in SPM. Moreover, Aβ (1-40) enhanced membrane lipid peroxidation. This study further confirmed that deposition of aggregated Aβ may be responsible for the alteration of phosphoinositides signalling in Alzheimer's disease.

CONTROL MECHANISMS OF THE RESPIRATORY ACTIVITY

Research team

Krystyna Budzińska, Beata Sokołowska

The study on early respiratory compensation mechanisms of the diaphragm dysfunction was continued.

1. Ventilatory responses to hypoxia and hypercapnia were studied before and after complete diaphragm denervation. The increments of tidal volume and of minute ventilation during hypoxia or hypercapnia were smaller after diaphragm denervation. Augmented breathing provoked by hypoxic stimulus never reached a volume of that in the intact animal in spite of comparable increase of the respiratory drive. Results suggest that activation of the rib cage muscles when the diaphragm is paralyzed is not sufficient to generate adequate lung ventilation. This ability attenuated with strenght of the chemical stimulus.

2. Experimental model of the diaphragm dysfunction was evaluated statistically. Part of the experimental data referred to above, was used to study whether a method of statistical pattern recognition based on k-NN rule (k-nearest neighbour) is proper to find most significant ventilatory parameters responsible for preserving the optimum level of the lung ventilation after interruption of the conduction in phrenic nerves. "Leave one out" method was used as a criterion for selection of those ventilation parameters. The study showed that the application of the standard k-NN rule, one of the most effective classification methods, allows finding the parameters that determine the general state of efficiency of respiratory system.

Short-term potentiation (STP) is one of the mechanisms supporting upper airway patency. This study tested the hypothesis that the excitatory aminoacid neurotransmission is involved in the short-term potentiation of hypoglossal activity due to laryngeal stimulation. Systemic administration

of an excitatory aminoacid antagonist, kynurenic acid, caused a transient depression of the hypoglossal activity. Both amplitude and duration of hypoglossal STP decreased. Alfa-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor antidesensitization agent, cyclothiazide, changed neither the respiratory pattern nor the response during laryngeal stimulation, however it prolonged the hypoglossal STP. The study suggest that changes in the excitatory aminoacid neurotransmission shape the hypoglossal STP. The phenomenon might be linked to desensitization of the AMPA receptor.

Cooperating units

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Publications

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PHARMACOLOGICAL NEUROPROTECTION IN CHOSEN VASCULAR-RELATED PATHOLOGIES OF THE CENTRAL NERVOUS SYSTEM

Research team

Paweł Grieb, Stanisław Chrapusta, Mirosław Ryba

Possible neuroprotective properties of an intracellular nitric oxide (NO) donor hydroxylamine have been assessed in the rat model of subarachnoid hemorrhage. It has been found that hydroxylamine treatment prevents the development of intracranial vascular angiopathy, as well as neurological deficits and beta-amyloid precursor protein (β-APP) overexpression.

In cooperation with the Department of Neuropathology, MRC, a series of experiments has been performed concerning the effects of CDP-choline, CMP and cytidine on the survival of CA1 hippocampal neurons in gerbils following short-term ischemia. Preliminary results suggest that cytidine alone displays similar neuroprotective potency as CDP-choline and CMP.

BIODEGRADABLE POLYMERS CONTAINING NUCLEOSIDE ANALOGS FOR TREATMENT OF INTRACRANIAL TUMORS

Supported by the State Committee for Scientific Research: grant # 4.P05F.024.12

Research team

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A preliminary assessment of the rate of release of a model nucleoside adenosine from a series of lactide-caprolactone and lactide-glycolide polymers was performed with the *in vitro* system modelling intracerebral conditions of polymer disintegration and convective and diffuse transport. Two

polymers capable to deliver micromolar concentrations of a nucleoside for over two weeks have been selected for further development.

MAGNETIC RESONANCE SPECTROSCOPY OF INTRACRANIAL TUMORS

Research co-ordinator

Paweł Grieb

In a collaborative project with the Institute of Oncology, Gliwice, a standardized methodology of acquisition and processing of proton magnetic resonance spectra from human brain *in vivo* with the use of Magnetic Resonance Tomograph Elscint Prestige 2 Tesla was developed. The methodology has been applied for the analysis of the spectra from normal human brains and from the areas of malignant gliomas. In gliomas an enhanced choline signal resulting in increased choline/aspartate and choline/total creatine metabolite ratios has been repeatedly found.

Cooperating units

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Publications

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LABORATORY OF PHARMACONEUROCHEMISTRY

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PHYSIOLOGICAL AND PATHOLOGICAL ROLE OF INTRACELLULAR CALCIUM IN BRAIN NEURONES

Research team

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Microdialysis of the hippocampus of postnatal day (PND) 7 rats *in vivo* was used in order to characterise a developmental aspect of previously described phenomenon of NMDA-evoked release of ^{45}Ca in the rat dentate gyrus (DG), reflecting mobilisation of intracellular Ca^{2+} *via* ryanodine receptors. Moreover, we tested a hypothesis that Ca^{2+} binding protein, calbindin D_{28K} may be involved in the mechanism of this effect. Application of NMDA induced a decrease in extracellular Ca^{2+} concentration and a masive release of ^{45}Ca to dialysate. Both effects significantly exceeded the adult levels. A specific binding of [^{3}H]ryanodine to receptors in DG and in the hippocampus CA3 sector was close to the adult level, whereas in DG of PND 7 rats only a slight expression of calbindin immunoreactivity was detected, contrasting with high level in the adult rats. These data do not support a hypothesis that calbindin may be involved in the mechanism of NMDA-evoked ^{45}Ca release, and point to significant participaton of the ryanodine-sensitive intracellular Ca^{2+} pool in generation of NMDA receptor-mediated calcium signal in the newborn rat brain.

Collaborative studies with the Open University, Milton Keynes, Great Britain, concerned the activity of voltage and excitatory amino acid receptor-operated calcium channels during induction and consolidation of memory traces. For this reason K^+ and excitotoxin-induced changes in intracellular concentration of Ca^{2+} were monitored in brain synaptoneurosomes of the PND 1 chicken after passive avoidance training. These experiments demonstrated potentiation of Ca^{2+} influx *via* voltage-dependent and NMDA channels 10 to 30 min after training. A ryanodine-sensitive intracellular Ca^{2+} pool also participated in NMDA-evoked increase in intracellular Ca^{2+} concentration. At later stages of the memory formation (3 to 6 hours after training) enhanced influx of Ca^{2+} *via* AMPA channels was noticed. These results confirm that excitatory amino acid receptors and calcium play a crucial role in the mechanisms of plastic changes in neurones during memory formatin.

Studies on the role of calcium-induced mitochondrial permeability transition (MPT) in brain neurones in the excitotoxic neuronal damage were undertaken in the collaboration with Department of Neuropathology, MRC. Electron microscopic studies demonstrated that a 20 min infusion of 1 mM NMDA *via* microdialysis probe to the rabbit hippocampus *in vivo*, leads to MPT in the pyramidal neurones of the hippocampal CA1 in the vicinity of dialysis probe. These changes, as well as calcium-evoked mitchondrial swelling of isolated rabbit brain mitochondria, were almost completely prevented by cyclosporin A. These results indicate that a method of brain microdialysis may be useful in studies of MPT in brain *in vivo*. In studies on the role of intracellular Ca^{2+} in the mechanism of conditioning to anoxia, it was demonstrated that a short, 2-min anoxia induces a biphasic increase in intracellular Ca^{2+} concentration, although of much lower magnitude than 10-min anoxia. The primary rise in intracellulr Ca^{2+}, evoked by 10-min anoxia, as well as a secondary increase in intracellular Ca^{2+} concentration during reoxyganation, were prevented by 2-min episode of pre-conditioning anoxia. Studies on the role of NMDA receptors in the process of adaptation to anoxia are in progress. (Collaboration with the Pavlov Institute of Physiology, St. Petersburg, Russia).

Our laboratory collaborated with the Laboratory of Experimental Pharmacology, MRC, in studies that demonstrated the protective effects of free radical scavenger, idebenon, on post-ischemic oxidative stress in brain of

rats submitted to cardiac arrest. Morphometric studies in this ischemic model, performed in collaboration with IBR, Staten Island, U.S.A. and Department of Neuropathology, MRC demonstrated biphasic neuronal loss in the rat hippocampal formation after 10-min cariac arrest. The results suggest that the lesion to CA1 neurones, observed already 3 days after insult, is a primary event triggering secondary damage to CA3 and DG neurones, fully developed after 14 days.

Cooperating units

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RELEASE OF EICOSANOIDS IN THE HIPPOCAMPUS *IN VIVO*: MODULATION BY NMDA RECEPTORS

Supported by the State Committee for Scientific Research: grant # 4.P05A.042.08

Research team

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Studies within the framework of this grant were intended to characterise a phenomenon of NMDA receptor-mediated release of prostaglandin D2 (PgD2) in the rat and rabbit hippocampus. In these studies we utilised *in*

vivo microdialysis combined with PgD2 radioimmunoassay in dialysates, and with monitoring disturbances of calcium homeostasis. Using these methods, we investigated dependence of PgD2 release on NMDA receptors, calcium signalling, and nitric oxide (NO) synthesis. Studies that were performed in 1998, conerned the dependence of the cyclooxygenase-mediated pathways of NMDA-evoked signalling in the rabbit hippocampus on NO. It was demonstrated that L-NAME, antagonist of NO synthase, inhibits NMDA-evoked NO release to dialysate, has no effect on NMDA-induced decrease in extracellular calcium concentration in the rabbit hippocampus, and inhibits in 30% NMDA-induced PgD2 release. This inhibition was reversed by L-arginine, but not by D-arginine. A spontaneous PgD2 production in the rabbit hippocampus was stimulated by L-NAME and by substances releasing NO, SNAP and sodium nitropruside. The obtained data indicate that a direct activation of cyclooxygenase by NO, described in other systems, concerns also rabbit brain neurones. These experiments completed the realisation of this grant.

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MODULATION OF TYROSINE KINASE CASCADE FUNCTION BY ISCHEMIA-INDUCED CALCIUM SIGNAL – AN INVOLVEMENT OF PROTEIN KINASE C

Supported by the State Committee for Scientific Research: grant # 6.P04A.010.14

Research team

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Pathological changes in the central nervous system (CNS) evoked by a variety of insults like ischemia, stroke, traumatic injury or neurodegenerative diseases are accompanied by neuronal apoptosis. Transient brain ischemia which induces massive apoptosis in hippocampal CA1 neurons causes a dual effect on protein kinase C (PKC). Previously we have shown that ischemic reaction consists of rapid enzyme translocation/activation, then by means of protease-dependent enzyme cleavage as well as the other factors, PKC becomes successively down-regulated. Concomitantly to suppression of the enzymatic activity, the decrease of PKC mRNA, especially γ isoform, was observed in brain vulnerable areas. The physiological significance of these sequential PKC changes, i.e. an initial activation and subsequent down-regulation, is still unclear. Since PKC activation is linked with an acute phase of ischemia, whereas, the enzyme down-regulation shortly proceeds or coincides with the onset of delayed postischemic neuronal death, we hypothesize that PKC inhibition rather than activation would be a critical factor in the induction of apoptosis.

A model study for the mechanism of cell death *in vitro* was established on neuroblastoma N2A cell line. By differential staining of living cells with Hoechst 33258 and Propidium Iodide, or by Terminal-dUTP-Transferase-Nick-End Labelling (TUNEL), transcriptional factor cJun/AP1 immunoreactivity and DNA laddering, we have shown that N2a cell line responded with classical apoptosis to PKC inhibition. Two different classes of PKC

inhibitors, staurosporine and GO6976, at concentration generally regarded as PKC-selective (10 nM and 0.5 μM, respectively) significantly increased the number of apoptotic cells in N2a cultures. The cells started to die at 2-3 hours after the treatment, then after 24 hours, approximately 70% of the cells acquired the apoptotic feature. This response was dependent neither upon the cell differentiation, nor PKC and bcl2 gene and protein expression but on the withdrawal of serum from growth medium concomitant to PKC inhibition. This effect of serum can not be mimicked or modified by selective suppression of Extracellular Signal Regulated Kinase (ERK) by treatment with Mitogen Activated Protein Kinase Kinase (MEK) – kinase inhibitor PD 98059. Thus, the apoptotic signal, acting when PKC-inhibited N2a cells are transiently deprived of serum nutrients, is totally independent from the parallel down-regulation of ERK pathway of Mitogen Activated Protein Kinases (MAPKs) cascade. The possible underlying mechanism(s) of PKC-inhibition induced apoptosis in N2a cells with special attention to Raf-1→Bcl2 protein interactions, are under further study in our laboratory.

Continuing our interest in ischemia-induced changes in protein composition of post-synaptic densities (PSD), we have estimated one of the most abundant protein with inducible tyrosine-kinase activity – the Focal Adhesion Kinase (FAK). This plasma membrane protein is functionally coupled with an integrin receptor and, as reported recently, can be activated by nerve cells depolarization and NMDA receptor activation. It was shown that PSD posses the highest concentration of FAK as compared with other membranes isolated from brain homogenate. Postdecapitative ischemia, although causing abundant FAK condensation in PSD, seems to depress rather than enhance this protein tyrosine phosphorylation/activation. Further experiments during postischemic reperfusion will be performed to gain more information about possible involvement of FAK in aberrant signalling initiated by cerebral ischemia.

CALCIUM-REGULATED SIGNALLING IN CEREBRAL ISCHEMIA

Research team

Krystyna Domańska-Janik, Agnieszka Bronisz-Kowalczyk, Barbara Zabłocka, Teresa Zalewska

Of the various enzyme systems activated by calcium, we have focused our studies this year on two functionally interrelated enzymes, rapidly activated by ischemic insult – protein kinase C (PKC) and neutral cysteine proteinase (calpain). Both enzymes are the major effector molecules mediating long-term effects in ischemia-induced cell death. Out studies show that their general reaction to ischemic stress have a lot in common. Initially, PKC and calpains are activated by facilitated interactions with their specific second messengers and translocated to highly specific regions of plasma membranes including post-synaptic densities (PSD). Then, the enzymes can interact with each other generating transient form of PKC (PKM), which is independent on the activators specific to the native enzyme. Then, both enzymes are deactivated by means of different routes. The mechanism of these biphasic responses and the duration of the activatory or inhibitory steps depend on ischemic model: activation processes (expressed by association with the plasma membranes and limited auto- or proteolysis) seem to predominate in the experimental model of transient, reversible ischemia of short duration while the global, irreversible ischemia results in persistent inhibition of all enzymatic activities. This may be related to the different mechanisms of neuronal cell death (necrosis *vs* apoptosis) induced in these two situations. By immunohistochemical methods we have confirmed apoptotic character of cell dying selectively in CA1 sector of hippocampus after transient cerebral ischemia of 5 min. duration. This reaction was preceded by activation of transcriptional factors like AP1 and NFκB. Substantial enhancement of AP1 binding activity measured by an Electrophoretic Mobility Shift Assay (EMSA) was observed in two different phases of postischemic recovery: at 3 hr after ischemia in the regions resistant to ischemic injury, then together with apoptotic reaction at 3-4 days after ischemia in CA1 hippocampus. The "supershift" test with antibodies anti phospho-cJun and cFos revealed differences in a dimmer composition of AP1 transcriptional factor at 3 hr and 3 days with cFos predominating in early phospho-cJun in late reactions after ischemia.

GENERATION OF A MULTI-POTENTIAL CELL LINE FOR QUANTITATIVE SCREENING FOR FACTOR INFLUENCING DIFFERENTIATION TOWARDS ONE OF THE MAJOR NEURAL LINEAGE

Research team

Krystyna Domańska-Janik, Leonora Bużańska

Recent reports have proven the existence pools of neural stem cells in adult brain with the ability to differentiate into either neurons, astrocytes or oligodendrocytes. These cells may thus constitute a potential source for endogenous repair in various pathological situations including brain or spinal cord ischemia, trauma, leucodystrophies, sclerosis multiplex etc. Before such therapeutical strategy reaches the level of clinic application there is a necessity to develop pharmacological tools that will allow these cells to proliferate and then, depending on involved pathology, to differentiate into particular types. As the step towards this goal, it seems crucial to develop a neural cell line as a tool for quantification of differentiating effects of various treatments into either neuronal, astro or oligodendroglial lines.

Cell line derived from a human medulloblastoma (DEV) isolated in the laboratory of dr. Belin in Lyon was chosen for experiments. The transfection with various developmentally specific transcription factors can drive the DEV cells towards a predominantly neuronal or glial phenotype. On the day of planting, the cells were transfected with the gene to be tested (inserted into an expression vector under CMV promoter) and co-transfected with CMV-GFP plasmid in 1/1 ratio. After 4 days the cells were immunolabelled with specific markers for neurons (TuJ1), astrocytes (GFAP) or oligodendrocytes (GalC). The results clearly demonstrate that the DEV cell line is suitable to be used for screening of agents that can influence its differentiation.

Cooperating unit

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Publications

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STUDIES ON MECHANISMS OF METABOLIC AND FUNCTIONAL CHANGES IN THE CENTRAL NERVOUS SYSTEM CAUSED BY TOXICITY OF LEAD

Research team

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Dopamine transport and dopamine D2 receptor binding

Lead (Pb) exposure is still a major medical problem both in environmental and occupational settings. In high doses, resulting in acute toxicity, Pb induces CNS damage known as lead encephalopathy with accompanying cerebral edema and hemorrhages. One of the acknowledged targets of Pb^{2+} is synaptic transmission. However, previous studies on the toxic effect of Pb on dopamine transport were few and fragmentary. Also, there is no information available regarding the effects of acute *in vivo* Pb toxicity in adult rats on dopamine transport and dopamine receptor binding in the brain.

Our results indicated that Pb intoxication causes an increased Pb level in blood and in the synaptosomal fraction and disturbs the transport of dopamine in synaptosomes. Uptake of dopamine to synaptosomes was inhibited by about 40%. Spontaneous release of dopamine from synaptosomes was very slow and strongly stimulated in the presence of veratridine. This

veratridine-dependent release of dopamine was inhibited in the Pb intoxication condition by about 80%.

Acute Pb treatment *in vivo* also affected the dopamine receptor D_2 by enlarging its affinity (reduced K_D) by about 51% and decreased density of receptor (B_{max}). In conclusion, results show that the exposure to acute *in vivo* Pb toxicity in adult rats causes disturbances in the CNS by interfering with the transport and receptor binding of dopamine, which is involved in the regulation of a variety of functions including locomotor activity, emotion and neuroendocrine secretion. It may lead to a severe instability of the CNS.

Myelin membranes morphology and activity of 2',3'-cyclic nuclectide 3'-phosphodiesterase EC. 3.1.437 (CNPase)

Up to now there has hardly been any credible information concerning the effect of Pb on the morphology and biochemical processes in the myelin of the CNS. Our results indicated that *in vivo* acute Pb intoxication drastically affects the morphology of myelin. The multilayered structure of myelin sheaths was regionally disturbed, with loosely arranged membranes or with ovoid-shaped swollen fragments. It was interesting to know whether the observed morphological disturbances are eflected by changes in activity of myelin marker – CNPase, the enzyme proven to be an integral protein of myelin.

Our experiments have shown, that acute Pb intoxication, carried on the rats *in vivo* caused significant inhibition of CNPase activity. The Michaelis-Menten Kinetics showed a decreased velocity and lower affinity of the enzyme. It seems unlikely that observed morphological abnormalities are the reflection of altered CNPase activity and kinetics but unquestionably it might be one of the possible reasons for these changes.

Cooperating unit

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MOLECULAR MECHANISM OF BRAIN AGING AND THE EFFECT OF AMYLOID BETA. ALTERATION OF SIGNAL TRANSDUCTION IN ISCHEMIC BRAIN

Research team

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To better understand the patomechanism of brain aging and ischemic encephalopathy the studies were continued on the molecular mechanisms of signal transduction in adult and aged brain and during reperfusion after brain ischemia.

Studies on the regulation of nitric oxide (NO) release in adult brain evaluated the mechanism of serotonin interference with the NMDA receptor dependent accumulation of nitric oxide (NO) and cGMP production. It was observed that serotonin through receptor $5HT_{1A}$ and $5HT_{1B/D}$ significantly influence the concentration of both NO and cGMP. It was found that agonists of the serotonin receptor $5HT_{1B/D}$ – zolmatriptan and sumatriptan are able to penetrate the blood brain barrier and protect the brain against overstimulation of NMDA receptor. In this way, the compounds may modulate the NO and cGMP dependent signal transduction pathway in the brain.

The role of cGMP dependent protein kinase G in the regulation of NOS synthase was examined. The results indicated that phosphorylation-dephosphorylation processes are important in the regulation of NOS in the brain. Inhibitor(s) of protein kinase(s) decrease NOS activity by about 20% and inhibitors of phosphatase(s) enhance the activity by about 20%. In continuation of studies on the molecular mechanism of post ischemia pathology the concentration of inositol phospholipid transport protein PI-TP was analyzed using monoclonal antibodies against PI-TP and Western blot technique together with the densitometric analysis.

Enhanced immunological reaction for both isoforms of PI-TP was found in the gerbil hippocampus at 15 min, 2 h and 7 days after 5 min brain ischemia. Simultaneously, a significantly lower immunochemical reaction for both PI-TP isoforms was found in the cerebellum. It appears that brain ischemia in gerbils leads to a significant of PI transport protein conformation and the response is different in different parts of the brain.

The alterations of conformation of these proteins may in consequence influence the function of PI-TP during reperfusion. These proteins are not only involved in PI transport but also in regulation of dynamics of cytoskeleton and vesicle transport of other molecules. The studies on PI-TP were carried out in cooperation with Dr G. Snoeck from the University of Utrecht.

In studies on molecular processes of brain aging, effect of amyloid beta peptides on the regulation of Ca^{2+} influx into brain synaptoneurosomes was investigated. The results confirmed our previous observations that aging does not disturb the function of voltage operated (VOCC) and NMDA receptor activated Ca^{2+} channels. Aggregated amyloid beta peptides, Aβ 25-35 and Aβ 1-40 μM enhanced the influx of Ca^{2+} through L and T type of VOCC at low depolarization condition up to 10 mM KCL. However Aβ peptides have no effect on the voltage operated Ca^{2+} channels in synaptoneurosomes depolarized with 75 mM KCL.

It was found that aggregated amyloid beta peptides also affected VOCC and other processes involved in regulation of cytosolic Ca^{2+} concentration in the brain cortex. Aggregated Aβ significantly inhibited the formation of inositol (1,4,5) trisphosphate, one of the most important messengers for Ca^{2+} mobilization. Non aggregated Aβ peptides have no effect on IP_3 release in brain cortex synaptosomes and brain slices.

Cooperating units

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Publications

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THE ROLE OF NEUROACTIVE AMINO ACIDS AND THEIR PRECURSORS IN HEPATIC ENCEPHALOPATHY AND AMMONIA NEUROTOXICITY

Research team

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The efflux of endogenous taurine, glutamate and glutamine was assayed by HPLC, and steady-state cell volumes were monitored with the [^{14}C] inulin method in rat cerebrocortical minislices in the absence or presence of 5 mM ammonium acetate ("ammonia") and/or inhibitors of osmosensitive amino acid transport: niflumic acid (NIF) and N-ethyl-maleimide (NEM). In the absence of ammonia, NIF abolished taurine efflux but did not affect glutamate or glutamine efflux and increased cell volume. NIF increased taurine, glutamine and glutamate efflux and increased cell volume. Ammonia strongly stimulated taurine (by 380%), and only moderately glutamate (30%) or glutamine efflux (76%), and increased cell volume. NIF inhibited, but did not abolish ammonia-dependent taurine and glutamine efflux, and did not change glutamate efflux. The effects of ammonia + NIF on cell volume did not differ from the effects of each compound separately. NEM inhibited ammonia-dependent efflux of all three amino acids, and NEM + ammonia decreased the cell volumes more than did each compound separately. It is concluded that although ammonia-induced taurine efflux is accompanied by an increase of cell volume, the underlying mechanism is not simply a cell volume regulatory response normally observed in hypoosmotic stress. Increased efflux of taurine, which is an inhibitory amino acid and a cell membrane protectant, may serve to counteract deleterious effects of increased excitatory transmission accompanying acute hyperammonemic insult.

Rats were treated with a hepatotoxin, thioacetamide (TAA) to induce hepatic encephalopathy (HE) and examined 21 days later, when they showed brain morphological changes indicative of excitotoxic neuronal damage, but not anymore biochemical or neurophysiological symptoms of HE. HPLC analysis of extracellular amino acids in striatal microdialysates of TAA-treated rats revealed a significant above control increase of basal levels of excitatory amino acids glutamate (Glu) and aspartate (Asp) and their amino acidergic metabolites glutamine (Gln) and alanine (Ala). TAA treatment triggered a 50 mM KCl ("high K^+")-dependent accumulation of Asp and Glu, and caused a decrease of Gln accumulation in the presence of high K^+, effect virtually absent in control rats. None of the treatments produces changes in accumulation of a nontransmitter amino acid leucine (Leu). The above changes mirror those previously described in symptomatic HE, and are likely to contribute to the excitotoxic damage. Basal microdialysate content of taurine (Tau), an inhibitory amino acid with antioxidant and volume regulatory properties, was 60% lower in TAA-treated rats than in control rats. The decrease of extracellular Tau may potentiate excitotoxicity, but on the other hand may manifest Tau redistribution to adjacent CNS cells, a process that serves various cell protective purposes. Stimulation with high K^+ increased extracellular Tau in control rats by 182%, and in TAA-treated rats by 322% above the basal level. Stimulation with 100 μM N-methyl-D-aspartate (NMDA) increased extracellular Tau in control rats by 27%, and TAA-treated rats by as much as 250% above the basal level. Only slight changes in the extracellular levels of the other amino acids were observed in the presence of NMDA in both control and TAA-treated rats. Enhancement of high K^+- or NMDA-dependent Tau release may manifest improved neuroprotective functions of the amino acid in rats with asymptomatic HE.

Acute hepatic encephalopathy (HE) is associated with disturbances in motor functions but the underlying mechanisms are not clear. Considerable experimental evidence suggests that the motor activity is modulated by striatal dopamine neurons whose discharge is under glutamatergic control, mostly through activation of the N-methyl-D-aspartate (NMDA) receptors. We used intrastriatal microdialysis to compare the effects of infusion of 10 mM NMDA, or 50 mM KCl as a generic release stimulus, on the extracellular levels of endogenous dopamine (DA) and its metabolites dihydroxy-

phenylacetic acid (DOPAC) and homovanillic acid (HVA) in control rats and in rats with acute HE induced with repeated administration of thioacetamide. HE significantly reduced the NMDA-dependent DA release but did not affect the KCl-induced release. The infusion of NMDA or KCL led to a decrease in extracellular DOPAC and HE did not modulate these effects. However, HE attenuated the NMDA- but not KCl-induced reduction in extracellular HVA. The basal levels of DA and DOPAC were not significantly altered by HE, while the HVA level was reduced. The results point to the impaired modulation of striatal DA discharge and metabolism by glutamate acting at NMDA receptors, contributing to the motor disturbances in HE.

In preliminary tests, C6 glioma cells in culture were found to actively synthesize a wide spectrum glutamate receptor antagonist, kynurenic acid (KYNA), which is believed to act neuroprotectively under conditions favoring glutamate neurotoxicity. Long-term treatment of the cells with 5-10 mM ammonia inhibited KYNA synthesis in these cells. Decreased KYNA production is thus likely to contribute to the excitotoxic nerve cell damage in hyperammonemia conditions *in vivo*.

GLUTAMINE TRANSPORT IN GLIAL CELLS DURING ACUTE OR CHRONIC EXPOSURE TO AMMONIA

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The uptake of radiolabelled glutamine to cultured C6 glioma cells was analysed in control conditions, with respect to the involvement of the individual transport systems known to carry out the uptake in other tissues. The study revealed the uptake to be mainly mediated by the N system, which in nontransformed astrocytes is responsible for glutamine outtransport from the cells. Other peculiarities of glutamine uptake in C6 cells include a relatively weak involvement of the L system, virtual absence of the sodium-dependent A system and a strong inhibition by homoserine.

Cooperating units

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POSTISCHEMIC CHANGES IN THE CENTRAL NERVOUS SYSTEM IN DIFFERENT EXPERIMENTAL MODELS

Research team

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In collaboration with the Laboratory of Ultrastructure of the Central Nervous System ultrastructural alterations of hypothalamo-neurohypophysial system have been analyzed in a new experimental model of clinical death - in rats with bilateral occlusion of the common carotid artery and 2 months survival after 10 min. cardiac arrest. In the supraoptic and paraventricular nuclei, severe ultrastructural changes took place including damage of neurosecretory neurons and their axons, activation of microglial and inflammatory cells, dysfunction of blood-brain barrier suggesting reduction in the secretory process. In collaboration with the Institute for Biological Sciences, National Research Council, Ottawa, Canada by means of 3 experimental models of brain ischemia in rats - bilateral occlusion of the common carotid artery, ligation of 4 arteries - carotids and vertebrals, and occlusion of the median cerebral artery, the neuroprotective effects of Coenzyme Q_{10} (CoQ_{10}) on postischemic distribution of chosen glucoconjugate receptors was shown with histochemical lectin technique. Alterations in localization of glucoconjugate receptors have been shown in 3 experimental ischemic models as compared with the control animals and those treated with CoQ_{10}. The histochemical distribution of RCA+ receptors in the capillary net, neurons and glial cells after ischemic insult was

dependent on the dose and time of CoQ_{10} application. In collaboration with the Department of Neurobiological Pathology, Institute of Brain Research in Developmental Disabilities in New York, studies on pathogenesis of juvenile neuronal ceroid lipofuscinosis and pathogenesis of cerebral beta amyloidosis have been performed. In the first topic, biosynthesis, intracellular localization and posttranslocation modification of the CLN_3 protein have been characterized. In the second topic, studies on human autopsy material in tissue culture and *in vitro* were performed. The results suggested different mechanism of development of beta amyloid deposits in arteries and capillaries and the same mechanism of development of beta amyloid in capillaries and diffuse plaques.

In collaboration with the Laboratory of Experimental Pharmacology and Department of Neurochemistry, MRC, changes in oxidative stress in the rat brain during reperfusion after cardiac arrest have been evaluated. The effect of treatment with free radical scavenger idebenone showed significant reduction of oxidative stress.

In collaboration with the Institute of Brain Research, Russian Academy of Sciences in Moscow and the Laboratory of Ultrastructure of the CNS, MRC, beneficial effects of GM1 ganglioside have been shown on photochemically-induced microvascular injury in cerebral cortex and hypophysis in rat. The same research team evaluated changes in capillaries of the rat brain in the model of focal cerebral necrosis. In human, histopathological changes have been correlated with immunohistochemical examinations using monoclonal antibodies to p53, bcl-2 proteins and CD-68 antigen in preoperative immobilisation of intracranial meningiomas to reduce tumor vascularity and blood loss during surgery. The relationship between necrosis within the embolised tumors and expression of two apoptosis-associated protein and macrophage/monocyte antigen was determined. The results indicated that expression of apoptosis-related proteins correlated with ischemic insult induced by preoperative tumor embolisation.

In collaboration with the Laboratories of Neurochemistry and Regulation of Transcription Processes of the M. Nencki Institute of Experimental Biology, we investigated the pattern of expression of interleukin-1beta (IL-1beta) and interleukin-6 (IL-6) immunoreactivities in rat hippocampus after transient complete brain ischemia evoked by cardiac arrest. Ischemic insult resulted in the concomitant and prolonged induction of both

interleukins in multiple astroglia especially in the CA1 area, most vulnerable to ischemia. Our data suggest that the astroglial IL-1beta and IL-6 may affect the neurodegradation of CA1 neurons and that the prolonged proinflammatory effects of IL-1beta prevail over the presumed protective action of IL-6.

The effect of CoQ_{10} on superoxide dismutase (SOD) activity, level of endothelin (ET)-1 in experimental models of cerebral ischemia in rat was investigated. ET-1 and ET-3 produced a regional specific decrease of SOD activity in the rat brain. As compared to ET-3, ET-1 produced longer lasting disturbances of enzyme activity. The beneficial effect of CoQ_{10} on recovery of SOD activity to the control values was observed. In ET-3 treated animals the above phenomenon was observed in the cerebral cortex and cerebellum after 4 h but after 24 h was still absent in the brain stem. Following ET-1 injection, treatment with CoQ_{10} produced recovery of SOD activity to the control level in investigated brain areas after 24 h.

VASCULAR AND VASCULOGENIC NEUROPATHOLOGICAL CHANGES IN BRAINS OF DECEASED AIDS PATIENTS

Research team

Irmina B. Zelman, Mirosław J. Mossakowski

We have performed preliminary morphological investigations of vascular and vasculogenic changes in 172 brains of patients, aged 23-64 years, who died in the course of AIDS in 1987-1997.

In 7 cases vascular malformations were found, among which only one (diffuse dysplasia of intracerebral vessels) influenced the neuropathology of the case being the source of multiple extensive blood extravasations. Among 54 cases with grossly visible necrotic/hemorrhagic lesions in 48 brains, they were associated with opportunistic infections, especially of necrotizing types such as toxoplasmosis, aspergillosis, cytomegaly, and/or with infiltrating malignant brain lymphomas. Vascular and vasculogenic pathology in the areas of tissue destruction of the above mentioned cases revealed great variability in individual cases reflecting on one hand the type of pathogenic agent and on the other hand the dynamics of the pathological process and the stages of its development. However, there were no

essential differences in the pattern of vascular and vasculogenic brain changes between AIDS and non-AIDS cases. It is worth mentioning that in cases with coexisting HIV-specific syndromes, the neuropathological expression of HIV-infection was usually increased in the vicinity of focal tissue destruction.

Grossly visible lesions of primary vascular origin were not a common phenomenon being recognized in our material only in 6 cases. Far more often micronecroses and small parenchymal foci of various size, intensity of tissue lesion and concomitant tissue reactivity were encountered. The neuropathology of these abnormalities corresponded generally to the pattern of postischemic changes. In the complicated pathomechanism of ischemic/hypoxic brain lesions in AIDS patients, several factors have to be taken into consideration, both systemic and intracerebral. Among intracerebral factors the following could be observed: structural changes in blood vessel walls with narrowing of vascular lumen, the presence of venous thrombi and diffuse intravascular coagulation causing occlusion of vascular lumen, microcirculation disturbances and increased blood vessels permeability.

In the broad spectrum of unspecific vascular wall abnormalities found in our material (focal swelling or necrosis, cellular and fibrotic hyperplasia, hyalinization, calcification etc.) it is worth mentioning focal cellular hyperplasia of small blood vessels named by us hypertrophic angiopathy. A characteristic feature of this vasculopathy was the abundant accumulation of small hyperchromatic cells with scanty cytoplasm within the changed part of vascular wall, leading to narrowing or even occlusion of vascular lumen and in some instances to the development of vasculogenic tissue necrosis. Sometimes among accumulated cells, nuclear conglomerates resembling the small HIV-multinuclear cells, were found. The origin of proliferating cells was not yet been established. It seems that they derive from histiocytes or other mononuclear cells integrally connected with the vascular wall, but their identification requires further investigations. We have selected in our material 31 cases with this peculiar type of vascular abnormalities. In 19 cases they were associated with the appearance of HIV-specific neuropathological syndromes. No relation was found between the presence of hypertrophic angiopathy and risk group of the patient or concomitant additional brain pathology. It seems that it is closely related to the

HIV activation in the brain and may precede the development of HIV-specific neuropathologic syndrome.

Morphological investigations of the frequency and characteristics of the opportunistic infections in the above mentioned Polish AIDS material revealed their presence in 57.5 percent of cases. The most common were CMV-infection (22.7%), toxoplasmosis (16.3%), progressive multifocal leucoencephalopathy (9.3%) and cryptococcosis (8.1%). The remaining viral, bacterial and fungal infections were present only in individual cases. It is worth mentioning 3 cases of brain aspergillosis and 5 cases of leptomeningeal tuberculosis, 3 of them occurred in the last year of observation.

FENOTYPIC AND GENOTYPIC EXAMINATION OF APOLIPOPROTEIN E ISOFORMS IN ALZHEIMER'S DISEASE AND AGING

Research team

Maria Barcikowska-Litwin, Maria Desperat, Marek Gołębiowski, Anna Pfeffer.

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. Recent studies have shown a strong association between the apolipoprotein E (APO E) ε4 allele in both sporadic, early and late onset of familial AD in different populations. Isoforms of APO E have been examined in a cohort of patients from an outpatient's clinic for Alzheimer's disease. The APO E genotyping has been performed according to the protocol of restriction isotyping by Chapman. APO E frequencies have been calculated by using gene counting method. Genotypes have been identified in persons with clinically diagnosed AD on the basis of DSM IV and NINCDS/ADRDA and NINDS/AIREN criteria. Our results did not differ from the results obtained elsewhere, and we observed a clear tendency to the more frequent presence of isoform ε4 within AD when compared with non demented cases. Allele ε4 of APO E appears in 40-50% of AD patients as compared with 10-20% in control cases. There has been no escalation in numbers of disease cases in 2/4 pairs alleles – which could suggest protective features of alleles 2. The females without Apo ε4 alleles progressed more rapidly (6.0 points per year on the Mini Mental Status Examination

(MMSE), 0.85 points per year on the GDS (Global Deterioration Scale). The rate of decline for females with other genotypes was slower at 0.92 points per year on the MMSE ($p<0.01$) and 0.5 points on GDS ($p<0.01$, and $p<0.05$ respectively). We suggest that APO E ε4 allele does not influence the age of onset and is not associated with more rapid course of AD. Indeed, it may even suggest a better prognosis in female AD patients. Our other data indicate significant correlation between decreased triglycerides and low density lipoprotein-cholesterol ratio in group of AD patients *versus* non demented individuals of similar age, independent of APOE genotype and increased total cholesterol and low density lipoprotein-cholesterol ratio in a group of AD patients, *versus* their children of the same genotype. In conclusion, the age factor has implications in serum lipid profile, that is independent from APOE genotype status in AD patients.

The search for APOE genotypes in AD cases in bigger cohort of patients (183 individuals) from central Poland has shown that the APOE ε4 frequency in Polish AD patients is similar to those reported from other Western countries and the APOE ε2 allele occurred with significantly lower frequency in AD patients than in age-matched controls. Moreover, APOE 4/3 and 3/3 had significantly higher frequency in AD than 4/4 genotype and there was no association either between age of onset and the number of ε4 alleles or specific genotype of Polish AD patients.

A volumetric MRI assessment of the hippocampal region was done in 50 patients with AD. The volumetric hippocampal measurements were 39.4% smaller in dementia of Alzheimer type than in controls. In differentiation between analyzed groups of patients the method had sensitivity of 95% and specificity 92%.

Cooperating units

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ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF THE BRAIN DEVELOPMENTAL DISORDERS. MAST CELLS IN CNS PATHOLOGY

Research team

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Microglial cells make up approximately 5% of the total glial cell population and are involved in the formation of the neuronal-glial microenvironment. In human development, relatively few studies have been performed on microglia. To achieve further insight into the participation of these cells in human brain development, microglial cells were examined by means of specific immunological markers in the cerebellum of fetuses, newborns and young children. Our study revealed for the first time that microglial cells changed their localization in developing cerebellum and participated in some events of the CNS development. They infiltrated the parenchyma of cerebellar cortex in a well-organized manner, and their migration was correlated with the development of some other cells in the cortex (eg. Purkinje cells). The results provided evidence that the localization of microglial cells in the cerebellum was precisely correlated with the appropriate stages of the brain development.

This year, mast cells were studied in human brains with congenital malformations of the vascular system. Mast cells are derived from precursors that originate in the bone marrow. These precursors circulate in blood and enter the tissues where their differentation and phenotype are modified by microenvironmental factors. Since, there are many reports linking mast cells with angiogenesis in developing tissue, the aim of the study was to examine the effect of different type of angiomas on maturation and phenotype of brain mast cells.The study was performed on brains with four major types

of angiomas of seventeen children and adults. Our results provided evidence that the differentiation of mast cell precursors in microenvironment of brain vascular malformations was similar as in normal brain of the control individuals. These cells were immunopositive with tryptase and chymase antibodies, thus they were tryptase-chymase phenotype (MC_{TC}). Additionally the increased number of mast cells were found within perivascular and interstitial parenchyma of angiomas, suggesting that these cells may play an important role in angiogenesis, in local homeostasis and/or inflammation. Some symptoms of the brain angiomas may be connected with degranulation of mast cells followed by delivery to the tissue, some biological active substances (histamine) which may affect the permeability of the blood brain barrier.

DEVELOPMENT OF THE NERVOUS SYSTEM AND PATHOLOGICAL PROCESSES OCCURRING IN THIS PERIOD

Research team

Maria Dambska, Izabela Kuchna*, Milena Laure-Kamionowska

The gradual maturation of morphological picture of meningoencephalitic inflammatory changes in children was observed in 20 fetuses and infants, as depending on tissue maturation and immunological response of the organism.

The coexistence of dysraphic malformations and holoprosencephaly was analyzed underlying the necessity for their classification of correlation between morphological picture and molecular and genetic data. Analysis of amyloid brain deposition in novel preseniline-1 mutation and a morphological study of olfactory neuroblastoma were additional studies realized in cooperation with units mentioned below.

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Cooperating units

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ULTRASTRUCTURAL, IMMUNOCYTOCHEMICAL AND HISTOCHEMICAL INVESTIGATIONS ON BRAIN VASCULATURE AND PERIVASCULAR SPACE IN BARRIER-COMPETENT AND BARRIER-FREE REGIONS. CONTINUATION OF THE STUDIES IN VARIOUS ANIMAL MODELS OF BRAIN ISCHEMIA

Research team

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We analyzed changes in vascular ultrastructure in the barrier-competent and barrier-free regions of rat brain after ischemia using different experimental models. Conventional electron microscopy, immunocytochemistry, and histochemistry were employed.

1. In the first set of experiments we evoked total brain ischemia by 10 min cardiac arrest according to the method described earlier by Korpatchev et al. (*Patol Fizjol Exper Ter* 1982, 3, 78-80). Immunocytochemical immunogold studies using antibodies against i-NOS and e-NOS were performed in order to study the localization and the kinetics of changes in labelling in the barrier-free region of rat brain. We showed that ischemia caused a 100-fold increase in expression of e-NOS in perivascular mast cells and a 10-fold increase in labelling in capillary endothelial cells. The peak of e-NOS expression was seen 3 hours after ischemia. Moreover, this is the first evidence of e-NOS expression in perivascular brain mast cells.

The expression of i-NOS was different from that of e-NOS and restricted to glia and perivascular and parenchymal macrophages.

We propose that e-NOS induced in perivascular mastocytes mediates NO production in the early post-ischemic period and thus constitutes an

important defense mechanism counteracting the effects of brain ischemia. In contrast to e-NOS, i-NOS is likely to cause damage in CNS during ischemia and therefore the observed expression of this isoform in glia and macrophages entering the brain may be deleterious rather than protective. It is thus conceivable that various types of NOS-producing cells within CNS may play different role in the pathogenesis of post-ischemic changes (protective for mast cells and endothelium and deleterious for glia and macrophages).

2. The reaction for NADPH-diaphorase was performed in the brain of rats subjected to total ischemia (according to Korpachev et al.) and additionally to traumatic brain damage. This histochemical assay has been used to determine NO production in tissues. We observed positive reactions in glial macrophagal cells in the perivascular and juxtaneuronal regions in both barrier-competent and barrier-free areas of the brain. This result supports our immunocytochemical data indicating that during ischemia glia produces NO *via* i-NOS.

3. In the models of focal brain ischemia evoked by photochemical vessel obliteration (platelet thrombi) or due to mechanical pressure, the ultrastructural changes were analyzed. In addition to endothelial damage, there were alterations in basement membrane and extracellular matrix. The basement membrane was thickened and multiplied and its normal layers were no longer recognizable. The repair reactions such as migration of pericytes and other cell types into damaged microvessels was seen. In addition immunofluorescence studies with antibodies against fibronectin and laminin were performed.

4. Employing the above described photochemical model of focal brain ischemia we analyzed the influence of GM1 ganglioside on the development of post-ischemic damage. GM1 was able to prevent formation of platelet thrombi and to reduce endothelial injury.

5. We analyzed late ischemic changes in the hypothalamo-neurohypophysial system in resuscitated rats with ligated carotid arteries and subjected to total ischemia according to Korpatchev et al. Electron microscopic assessment was performed at 2 months of survival. Ultrastructural changes suggesting dysfunction of the hypothalamo-neurohypophysial system were observed. This experimental model may thus be utilized in research on pathogenic mechanisms of post-ischemic encephalopathy.

THE ROLE OF IONIZED CALCIUM AND CALCIUM CHANNELS IN APOPTOSIS IN NORMAL AND CANCER CELLS. EXPERIMENTS USING FLUORESCENT CALCIUM DETERMINATION IN LIVING CELLS AND ELECTRON MICROSCOPY

Supported by the State Committee for Scientific Research: grant # 4.PO5A.045.15

Research team

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The aim of the project is to characterize the role of calcium in the processes of programmed cell death due to terminal differentiation or apoptosis. The ultrastructural changes in the cytoplasm, plasma membrane and nucleus will be correlated with activation of specific intracellular signalling pathways. The project was commenced in November 1998. Since then we have succeeded in the development of a new method, so-called embedment-free electron microscopy which is a new technique enabling ultrastructural studies of nuclear matrix. Moreover, fluorescent techniques characterizing chromatin structure and conformation were optimized. Studies on the influence of calcium as a death signal on the structure of chromatin and nuclear matrix are about to be started.

CHANGES IN KERATINOCYTE ULTRASTRUCTURE IN DIFFERENT PATHOLOGICAL CONDITIONS

KH 1060 is a vitamin D analogue causing epidermal hyperplasia in topical application. We showed that this compound disrupts a calcium gradient which is normally present in epidermis and responsible for epidermal cell growth control.

It has been shown that morphological changes in dying keratinocytes strongly depend on the factor inducing apoptosis.

ULTRASTRUCTURAL AND HISTOCHEMICAL STUDIES IN HYPOXIC ISCHEMIA IN NEONATAL RATS

We describe the ischemia-induced changes in calcium distribution using the oxalate-pyroantimonate method for electron microscopy.

Cooperating units

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Institute of Anatomy and Cell Biology, University of Göteborg, Sweden (M. Puka-Sundvall, H. Hagberg)

Department of Cardiology, The Hospital of the Ministry of Internal Affairs, Warsaw, Poland (M. Dąbrowski)

Department of Surgery, Medical School, Warsaw, Poland (J. Polański)

Medical Research Centre, PASci, Warsaw, Poland:

- Department of Signal Transduction (J. Strosznajder)
- Department of Neuropathology (A. Kapuściński, M. Śmiałek)
- Laboratory of Pharmaconeurochemistry (J. Łazarewicz, W. Gordon-Krajcer, M. Alaraj)
- Laboratory of Molecular Neuropathology (K. Domańska-Janik)
- Laboratory of Pathobiochemistry (U. Rafałowska)
- Department of Neurophysiology (M. Pokorski)
- Department of Neurotoxicology (J. Albrecht)
- Laboratory of Neuropeptides (A. Lipkowski)

Publications

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EVALUATION OF INTRACRANIAL HOMEOSTASIS DISTURBANCES: EVALUATION OF DISTURBANCES OF INTRACRANIAL VOLUME COMPENSATION, OF CEREBROVASCULAR REACTIVITY AND OF NEUROPHYSIOLOGICAL DISTURBANCES IN PATIENTS WITH FOCAL CNS LESIONS

Research team

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Department of Neurosurgery participates in the realization of 2 scientific grants: one in cooperation with the State Committee for Scientific Research and the other with the Polish Science Foundation. Studies performed concerned 3 main topics: cerebrovascular reactivity, disturbances of intracranial volume reserve and electrophysiological disturbances. Cerebrovascular reactivity was investigated experimentally and clinically. New methods for digital evaluation of Doppler signal were developed. Possibilities of intraoperative application of Doppler microprobe to evaluate local circulation were further studied. Other aspects of Ca-channel blocker Dotarizine activity were investigated in experiments on rabbits. Its stabilizing effect upon the vascular reactivity was observed which might make the application of Dotarizine in the migrene treatment a promising perspective.

Evaluation of cerebrovascular changes during neurophysiological testing was continued in the clinical studies. Individuals with dyslexia and normotensive hydrocephalus were investigated. A distinct pattern of functional lateralization in the dyslexia group and changed profile in the task performance combined with the decrease of cerebral blood flow in left medial cerebral artery in the normotensive hydrocephalus group were found. Results of the previous experiments with the Transcranial Doppler Sonography (TCD) applicability in patients after head injury were published. An important role of TCD in evaluation of cerebrovascular reactivity disturbances and vasospasm development was emphasized. In the field of intracranial volume-pressure relation disturbances studies concerning differential diagnosis of hydrocephalus were continued. Sixty five new cases of hydrocephalus were evaluated. Hydrodynamic features of hydrocephalic valves were investigated in cooperation with the Centre in Cambridge. Methods of *in vitro* and *in vivo* valve testing have been developed. The goal of the study is also to formulate indications for implantation of a certain shunt type in a specific hydrocephalus type.

Experimental studies concerning applicability of physiological loadings in evaluation of the intracranial volume reserve are continued. In the reported period 16 experiments have been performed. The study will be completed next year. Electrophysiological studies were conducted in cooperation with the Nencki Institute of Experimental Biology. It was found that removal of cerebellar cortex in cats led to somatosensory evoked potentials (SEP) increase and the removal of the whole cerebellar hemisphere caused SEP decrease and occurrence of characteristic oscillations. Identical oscillations were observed in patients with cerebellar hemisphere lesions.

Along with the planned scientific activities, studies concerning neurooncology were conducted. Anatomical aspects of a surgical approach *via* the fourth ventricle floor to brainstem tumors are a subject of the doctor's dissertation that is being reviewed. Correlation between high-resolution proton spectroscopy picture and malignancy grade of the tumor will be investigated. Thirty specimen samples have already been processed. Another doctorate is being supported by the State Committee for Scientific Research and conducted in cooperation with the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences. Thanks to this grant, another doctorate work concerning T3 and retinoid nuclear receptors

expression in gliomas has been started and 6 intraoperatively obtained samples are being processed. Publications on the clinical topics concern problems like aneurysm embolization with detachable coils or suboccipital neuralgia as a complication of operation in the suboccipital region.

METHODS OF MEASUREMENT AND EVALUATION OF THE MECHANISMS OF CEREBRAL BLOOD FLOW CONTROL

Supported by the State Committee for Scientific Research: grant # 8.T11E.005.14

Research co-ordinator

Zbigniew Czernicki

According to the schedule a method of cerebrovascular reactivity evaluation was elaborated.

Cooperating unit

Academic Neurosurgical Unit, Addenbrooke' Hospital, Cambridge, UK (M. Czosnyka)

IN VITRO MR SPECTROSCOPY OF HIGH RESOLUTION IN INTRACEREBRAL TUMORS TISSUE AND CEREBROSPINAL FLUID

Supported by the State Committee for Scientific Research: grant # 4.P05B.054.14

Research team

Zbigniew Czernicki, Dariusz Horsztyński, Jerzy Walecki

Neoplastic tissue samples from 40 patients were collected intraoperatively. They were evaluated by means of MR proton spectroscopy in order to find possible correlation between characteristics of spectroscopic spectra and tumor histology.

EXPRESSION OF NUCLEAR T3 AND RETINOID RECEPTORS IN GLIOMAS

Supported by the State Committee for Scientific Research: grant # 4.P05C.005.15

Research team

Zbigniew Czernicki, Paweł Nauman

Neoplastic tissue samples from 12 patients were collected intraoperatively. Proteins were fractionated using Western blot system. Blotting was performed using antibodies against Trα1, Trα2, Trβ1 protein receptors.

NON-INVASIVE METHOD TO EVALUATE HEMODYNAMIC CHANGES AND AUTOREGULATION IN SEVERE BRAIN TRAUMA AND CEREBROVASCULAR DISEASES

Supported by PHARE-SCI-TECH II: grant # PL9611-03-02

Research team

Jerzy Jurkiewicz, Zbigniew Czernicki, Katarzyna Jarus-Dziedzic, Elżbieta Łuczywek, Wojciech Sapieja

According to the plan assumptions that comprize minimal and optimal requirements for functions evaluated by the diagnostic system were developed.

Cooperating units

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Publications

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STUDIES ON THE STRUCTURE AND FUNCTION OF MUSCLE AND NERVE DURING DEVELOPMENT, AGING AND GENETIC AND ACQUIRED DISEASES

Research team

Irena Hausmanowa-Petrusewicz, Małgorzata Dorobek, Hanna Drac, Anna Fidziańska, Hanna Jędrzejowska, Anna Kamińska, Irena Niebrój-Dobosz, Janina Rafałowska, Katarzyna Rowińska-Marcińska

During the past year, the main task of muscle research group was to apply the methods of molecular genetics to the main research problem. It was done either by the introduction of new genetic methods or by intensive collaboration with other genetic centers. This application mostly concerns Duchenne (and Becker) dystrophy, Emery-Dreifuss dystrophy, spinal atrophy and some neuropathies. Dystrophinopathy in females was studied and led to the description of two groups - familial and isolated carriers. The laboratory tests in these groups indicated a high activity of creatine kinase, electromyographic abnormalities, a dystrophic pattern of muscle biopsy and a mosaic distribution of dystrophino-positive mixed with dystrophino-negative muscle fibers. Western blots revealed a fall in dystrophin abundancy and/or decrease of molecular weight. These findings are important for genetic counselling and a proper diagnosis of dystrophic females previously misdiagnosed as limb-girdle dystrophy. Important findings were obtained in collaboration with E. Hoffman (Pittsburgh) on the dystrophin missense mutation showing a persistence of dystrophin in spite of the typical, severe course of Duchenne muscular dystrophy. This is a unique case in myological literature and it is very important theoretically and also from a practical point of view.

In Emery-Dreifuss besides the X-linked type of disease, the second form inherited as a dominant autosomal trait was distinquished – this new defi-

nition was possible due to a collaboration with the Pavia Genetical Center of Molecular Genetics.

Molecular diagnostics and correlation of phenotype-genotype was studied in demyelinating neuropathies (type I Charcot-Marie-Tooth) and the duplication was localized.

Spinal muscular atrophy (SMA)

Experimental aging in rats and regeneration in very old rats was studied. The apoptosis in muscle and spinal cords of SMA fetuses and in the muscle of SMA infants was looked at – it is the start of a new project in the framework of the SMA problem. A separate group of studies concerns electrophysiology. The main interest was the irregularity and complexity of motor unit potentials – satellite potentials, double discharges and the diagnostic yield of these potentials in neuromuscular disorders.

Cooperating units

Department of Molecular Genetics, Pittsburgh, USA (E. Hoffman).
Institute of Genetics, Pavia, Italy.
Institute of Pharmacology, Berlin, Germany.
Institute of Pharmacology, Kraków, Poland.
Department of Genetics, Medical School, Poznań, Poland.
Department of Neurology, Medical School, Warsaw, Poland (E. Zalewska).

GENETIC AND CLINICAL STUDIES ON INFANTILE AND JUVENILE SPINAL MUSCLE ATROPHY (SMA) – CORRELATION BETWEEN PHENOTYPE AND GENOTYPE

Supported by the State Committee for Scientific Research: grant # 4.P05E.001.12

Research team

Irena Hausmanowa-Petrusewicz, Maria Jędrzejowska, Anna Fidziańska, Irena Niebrój-Dobosz

Molecular tests for SMA detection were introduced in many centers around the country. The aim of the test was, first of all to detect the possible deletion of the SMN (survival of motor neuron) gene.

The collection of a very large amount of materials from the whole country permits the analysis of the correlation between phenotype and genotype to evaluate the course of the disease and of atypical cases. It was found that 10-15% of severely affected children with SMA type 1a can even survive a long time, which changed the prognosis in this type of disease. In more than 100 infants suspected of SMA deletion of exons 7 (and sometimes also 8) was found, in some cases this finding was associated with deletion of gene NAIP (neuronal apoptosis inhibitory protein) and p22. Very large deletions were usually associated with severe clinical conditions.

We are performing prenatal diagnosis and are trying to introduce the detection of carriership of SMA.

Cooperating units

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TUMOR NECROSIS FACTOR-α (TNT-α) INDUCED PATHOLOGY IN RAT BRAIN: CHARACTERIZATION OF STEREOTAXIC INJECTION MODEL

Research team

Janina Rafałowska, Stanisław Krajewski

Stereotaxic administration of TNF-α *in vivo* into rat brain produces a disturbances of the BBB permeability, infiltration of the injected area by blood derived macrophages and early arising astrogliosis. These changes were considered as manifestation of the role of TNF-α in inflammatory processes.

Cooperating unit

Department of Neurology, School of Medicine, Łódź, Poland (A. Głąbiński)

DOES THE PATHOLOGICAL FACTOR IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) ALSO DAMAGE ASTROCYTES?

Research co-ordinator
Janina Rafałowska

Evaluation of the anterior horn of spinal cord in 11 sporadic ALS cases showed very intensive neuronal changes associated with weak astrocyte reaction. The influence of an unknown pathological factor on both motoneurons and astrocytes was suggested.

Cooperating unit
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HIV-1-INFECTION IN THE CNS. A PATHOGENESIS OF SOME NEUROLOGICAL SYNDROMES IN LIGHT OF RECENT INVESTIGATIONS

Research co-ordinator
Janina Rafałowska

The main factors relevant for the pathogenesis of AIDS-dementia complex (ADC) may be classified to two groups. The "nonspecific" factors are present in each immunologic process manifested by inflammation. The second group may consist of factors connected with HIV-1 infection of CNS: gp 120, gp 41, nucleotide sequence variability, a variety of virus replication in several parts of the CNS and a presence of co-receptors to HIV-1 virus.

VASCULAR CHANGES IN TUBERCULOUS MENINGOENCEPHALITIS

Janina Rafałowska

Two cases of tuberculous encephalomeningitis which differ considerably in the course of the disease, as well as in pathological changes, were reported. A different morphological picture probably depends on the type and virulence of *Mycobacterium tuberculosis* and on the host immune response to the infection.

Cooperating unit

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Publications

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IMMUNOLOGICAL MARKERS IN DIAGNOSIS AND THERAPY OF MULTIPLE SCLEROSIS (MS)

Research team

Mieczysław Wender, Jacek Losy, Grażyna Michałowska-Wender, Elżbieta Tokarz-Kupczyk

Platelet-endothelial cell adhesion molecule (PECAM-1) is a 130-kDa glycoprotein, member of the immunoglobulin superfamily, which is involved in transendothelial migration of leukocytes. We presented data showing that soluble PECAM-1 is significantly elevated in sera of patients with active gadolinium enhancing lesions. As we have found increased levels of soluble PECAM-1 in sera of patients with active magnetic resonance imaging (MRI) lesions, but not in patients without enhancing lesions, the molecule may be regarded as marker of MS activity. Soluble PECAM-1 may be involved in immunomodulatory mechanism leading to inhibition of transendothelial migration of leukocytes in MS.

Fifty two clinically confirmed MA patients were treated by subcutaneous injections of 5 mg 2-CDA (cladribine) in 5 consecutive days. The injection courses were repeated 6 times at one month intervals. The MRI pattern and immunological markers were studies in serum and cerebrospinal fluid (CSF) before and after 6 months treatment. The obtained results suggest that treatment with 2-CDA has no significant effect on humoral immunological events in MS, which is in contrast to some normalization of cellular immunological processes.

The evaluation of free light chains kappa in urine was performed in 77 cases of MS, including 52 patients before and after treatment with 2-CDA and in 25 patients before and after therapy with high doses of Prednisone. The high variations in the level of free kappa chains indicate limited diagnostic value limited to cases with a very high level. In chronic progressive

MS group we have found the effect of 2-CDA therapy on free kappa light chain value. The significant effect of Prednisone treatment was observed in early onset cases of MS and in cases with clinical improvement after therapy. In conclusion, the study suggests that urinary free light chains level may be considered as one of the markers for monitoring of the effect of therapy on the activity of the immunological processes in MS.

Cooperating unit

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GENETIC STUDIES OF ALZHEIMER DISEASE

Research co-ordinator:
Mieczysław Wender

Recent data have demonstrated that the presence of apolipoprotein E (APOE) ε*4 allele is a major risk factor of Alzheimer disease (AD). We determined the APOE genotype in 64 patients with sporadic AD and in 43 non-demented aged controls selected from Poznań region and the western part of Poland. Study was performed using polymerase chain reaction (PCR) followed by the restriction fragment length polymorphism analysis. We confirmed a strong correlation of the APOE ε*4 allele with sporadic late-onset AD. In contrast to many previous reports we did not find an association between the APOEε*4 allele and sporadic early-onset AD.

Recently, an association between the intronic polymorphism in presenilin-1 (PS-1) gene and late-onset AD was claimed. In order to confirm this observation we studied a sample of Polish patients with sporadic AD. Our results did not confirm the existence of an association between the intronic, polymorphism in the PS-1 gene and late-onset AD.

The majority of early-onset familial Alzheimer disease (EOAD) has been associated with mutations in a novel gene on chromosome 14 which has been termed presenilin-1 gene. We screened for mutation within the presenilin-1 gene in twenty patients with EOAD using a PCR-SSCP analysis. We found three aberrant (mutant?) band pattern for exons 4 and 7 in three unrelated patients.

Cooperating unit

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Publications

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THE EFFECTS OF REDUCED OR ENHANCED LEVEL OF PHYSICAL ACTIVITY AND ONTOGENIC DEVELOPMENT ON PHYSIOLOGICAL RESPONSES TO VARIOUS STIMULI

Influence of short-term bed rest on hemodynamic, metabolic and neurohormonal responses to glucose load in athletes and sedentary men

Research team

Krystyna Nazar, Hanna Kaciuba-Uściłko, Andrzej W. Ziemba

Eleven untrained students, 8 long distance runners and 10 strength trained athletes were examined before and after 3-day bed rest (BR). Blood glucose (BG), plasma insulin (IRI), noradrenaline (NA), adrenaline (A), heart rate (HR), and blood pressure (BP) were measured before and during 2 hrs following glucose (75 g) ingestion. BR markedly increased IRI response in all subjects, while BG response was elevated only in sedentary men. The greatest increases in IRI and IRI/BG ratios were found in endurance athletes. The data from all subjects ($n = 29$) revealed that the initial plasma NA and glucose-induced increases in NA and A were lowered after BR ($p<0.01$). These effects were most pronounced in endurance athletes. BR did not influence HR or BP. It is concluded that (1) the athletes have more adequate compensation for the BR-induced decrement in insulin sensitivity than sedentary men; (2) three-day BR diminishes basal sympathetic activity and attenuates sympathoadrenal activation induced by oral glucose; and (3) endurance athletes show greater sympathetic inhibition than strength athletes or sedentary men.

Cooperating units

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Effects of three-day bed rest on physiological responses to graded exercise in the endurance-trained athletes and in sedentary men

Research team

Krystyna Nazar, Hanna Kaciuba-Uściłko, Barbara Bicz

Twelve healthy, male untrained students and 10 endurance trained athletes (cyclists) volunteered for this study. Before and after 3-day bed-rest (BR) they were submitted to graded bicycle exercise test. BR reduced maximal work load and VO_2max with a tendency towards augmentation of pulmonary ventilation. These effects were more pronounced in athletes than in sedentary subjects. Only in cyclists blood lactate threshold was shifted to lower work loads. In both groups, pre- and post-exercise plasma renin activity was ele-vated, plasma growth hormone concentrations were diminished and plasma noradrenaline threshold was lowered after BR. Plasma cortisol levels were increased only in cyclists. It is concluded, that 3-day BR diminishes aerobic capacity and work tolerance, modifying also some hormonal responses to exercise. Most of these effects are more pronounced in athletes than in sedentary men.

Cooperating units

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Cardiovascular, metabolic and plasma catecholamine responses to passive and active exercise

Research team

Krystyna Nazar, Gerard Cybulski, Barbara Kruk, Krzysztof Krzemiński, Wiktor Niewiadomski, Andrzej W. Ziemba

The aim of this study was to investigate contribution of muscle mechanoreflexes to the cardiovascular and sympathoadrenal responses to dynamic exercise. Hemodynamic, metabolic and plasma catecholamine changes were compared during active unloaded and passive cycling. An increase in heart rate and a decrease in total peripheral resistance were found only during active exercise, while the significant increases in stroke volume and blood pressure occurred only during passive cycling. The increases of cardiac output, shortening of preejection period (PEP) and a decrease of PEP to ejection time ratio were similar in both types of exercise. A tendency towards an elevation of plasma noradrenaline was found only during active exercise. The results suggest that muscle mechanoreceptors are not engaged in chronotropic response of the heart to dynamic exercise, but they contribute to the enhancement of cardiac contractility.

Resting metabolic rate and thermogenic effect of glucose in trained and untrained girls aged 11-15 years

Research co-ordinator

Krystyna Nazar

Twelve girls involved in regular training with the specialization of rowing were examined twice a year for 4 consecutive years. Their initial age was 11.7 ± 0.2 years. Control groups consisted of 13 girls aged 11.5 ± 0.3 years and 18 girls aged 14.4 ± 0.3 years examined simultaneously with trained ones in the first and last year of the study, respectively. The study included basic anthropometry, estimation of sexual maturation, 2-day dietary reports, measurements of resting metabolic rate (RMR), thermogenic effect of glucose (TEG), blood glucose (BG) and plasma insulin (IRI) concentrations. Body mass, height and fat content were slightly greater in trained than in untrained girls. None of the subjects had any disturbances in men-

strual function and the age of menarche in trained girls was close to that in untrained subjects. Girls from both groups reported similar daily energy intake close to the lower limit of the FAO/WHO/UNU estimates of energy requirements. RMR per kg of total body mass or mean body mass were significantly lower in trained than in sedentary girls ($p<0.001$ and $p<0.01$, respectively) while TEG was greater in the former ($p<0.05$). Plasma IRI after glucose ingestion was lower in trained than in untrained girls. The results suggest that in circumpubertal girls, increased physical activity leads to energy conservation at rest in postabsorptive state and a tendency towards enhancement of food-induced thermogenesis.

Cooperating unit

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Comparison of aerobic and anaerobic capacity of teenagers practicing swimming or athletics with age matched groups of subjects not involved in sport

Research co-ordinator

Krystyna Nazar

Laboratory and field exercise tests were performed in 472 boys and 417 girls aged 11-15 years. Swimming and athletic training, applied in the sports-directed schools, significantly increased aerobic performance (VO_2max) being less effective in development of anaerobic fitness (maximal and mean power during 30 s Wingate test). Results of the standard field tests (running, jumping and throwing) and those of Wingate test significantly correlated with the plasma testosterone concentration in boys which indicates an important role of this hormone in anaerobic fitness development.

Cooperating unit

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EFFECTS OF DIETARY CREATINE SUPPLEMENTATION ON EXERCISE PERFORMANCE IN ATHLETES AND ON MUSCLE ENERGY SUBSTRATE CONTENTS IN RATS

Influence of creatine supplementation during endurance training on aerobic and anaerobic performance in elite rowers

Research team

Krystyna Nazar, Jolanta Chwalbińska-Moneta

Creatine monohydrate (20 g daily) or placebo were administered, in a double blind manner, to 16 elite rowers during 5 days of intensive endurance training. It was demonstrated that a short creatine supplementation significantly shifts the blood lactate thresholds towards higher work loads in comparison with placebo group. Creatine ingestion caused also prolongation of the anaerobic supramaximal exercise (7 W/kg body mass) and elevation of blood lactate concentration achieved during this effort.

Effect of a short-term dietary creatine supplementation on high-energy phosphates in the rat myocardium

Research team

Krystyna Nazar, Zofia Brzezińska, Ilona Falęcka-Wieczorek, Hanna Kaciuba-Uściłko

The aim of this study was to find out whether creatine feeding affects total creatine, phosphocreatine (PCr), adenine nucleotide contents and β-hydroxy-acyl-CoA-dehydrogenase (HAD) activity in myocardium as compared to red skeletal muscle. Ten adult Wistar rats received creatine (2.5 % of diet weight) for 7 days. In creatine fed rats, PCr was increased (by approx. 20%) in cardiac and in soleus muscles with ATP elevated in myocardium and total and free creatine in soleus. In both muscles, creatine feeding enhanced HAD activity. It is concluded that dietary creatine does increase cardiac muscle high energy phosphate reserves and its oxidative potential.

Cooperating unit

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LIPID METABOLISM IN SKELETAL MUSCLES

Effect of fatty acid availability on glucose utilization in rat soleus muscle *in vitro*

Research team

Leszek Budohoski, Monika Górecka, Anna Dubaniewicz, Marcin Synak, Ewa Żernicka

The aim of this investigation was to determine the effect of various concentrations of palmitic acid in the medium on insulin-stimulated glucose utilization by rat soleus muscle. The rates of 2-deoxyglucose (2DG) transport to muscle cells and lactate (LA) formation in the soleus muscle were measured *in vitro* at 100 $\mu U \times mL^{-1}$ insulin concentration. Only a slight decrease (NS) in the rate of 2DG transport to muscle cells and unchanged rate of LA production were noted when palmitate concentrtion in the medium increased from 0.5 to 2.0 mM. These data indicate that under *in vitro* conditions, glucose transport and utilization by soleus muscle are not inhibited even by high (2mM) concentration of palmitate.

Hormone-sensitive lipase (HSL) expression and regulation in skeletal muscle

Research team

Leszek Budohoski, Józef Langfort

Expression of immunoreactive HSL in rat skeletal muscles was evidenced using the Western-blotting technique of isolated muscle fibers. It was found that the enzyme content is higher in the oxidative than in glycolytic fibers and that it is activated simultaneously with glycogen phosphorylase during muscle contractions. HSL activity could be abolished by antiserum against HSL. It was also demonstrated that muscle HSL is stimu-

lated by adrenaline through the β-adrenergic mechanism involvin cAMP-dependent protein kinase.

Cooperating unit

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Muscle lipid metabolism during cold-acclimation in female rats

Research team

Leszek Budohoski, Zofia Brzezińska, Hanna Kaciuba-Uściłko, Krystyna Nazar, Ewa Żernicka

The aim of this study was to find out whether thermogenesis in skeletal muscles plays a role in adaptation to cold in rats. For this purpose, in preliminary experiments muscle triacylglycerol (TG), total, free, and acyl carnitine contents, muscle cytochrome c oxidase (COX) activity as well as plasma TG and free fatty acid (FFA) concentrations were determined in three groups of Wistar female rats (8 animals per group): (1) Controls (kept at thermoneutrality), (2) Rats acclimatized to 6 ^{0}C for 6 weeks, and (3) Cold-acclimatized rats pair-fed with controls during the last 12 hours before sacrifice. In comparison with controls, in the cold-acclimatized rats, plasma FFA and TG concentrations were decreased, while muscle total and particularly acyl carnitine as well as activity of COX were enhanced. These findings suggest enhanced lipid utilization by skeletal muscles in rats adapted to cold.

Cooperating unit

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EFFECT OF OCCUPATIONAL WORK ON CARDIOVASCULAR, HORMONAL AND METABOLIC INDICES IN PATIENTS WITH CHRONIC CIRCULATORY AND METABOLIC DISEASES

Supported by the Strategic Governmental Program:
„Labor safety and health protection in work environment", grant no 04.10.6

Research team

Krystyna Nazar, Jolanta Chwalbińska-Moneta, Gerard Cybulski, Hanna Kaciuba-Uściłko, Barbara Kruk, Hubert Krysztofiak, Krzysztof Krzemiński, Wiktor Niewiadomski, Andrzej W. Ziemba.

The aims of this study are (1) to assess the effects of occupational stress on cardiovascular, hormonal and metabolic indices in patients with coronary heart disease, hypertension, diabetes and obesity, (2) to adapt a model of laboratory tests for prediction of the patients' responses to real life stress occurring in occupational work, (3) to elaborate a system of evaluation of health risk connected with occupational work in patients with chronic circulatory and metabolic diseases

In the first year of this project the groups of 182 patients and 10 healthy men who assessed their occupational work as stressful, were recruited for the study basing on medical records, profession and age. The preliminary recordings of 24 hour ambulatory ECG and blood pressure were performed and the questionnaires for estimation of psycho-social work conditions and the subjects' mood profiles were tested.

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Publications

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ROLE OF EICOSANOIDS IN REGULATION OF RENAL EXCRETION: INVOLVEMENT OF CYTOCHROME P450 ENZYMES?

Research team

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The mechanism of natriuresis and diuresis in response to extracellular volume expansion, e.g. resulting from intravenous saline loading, has not been satisfactorily elucidated. Multiple alterations, physical, humoral and neural, have been invoked but the relative contribution of individual factors proved difficult to delineate. One of the reasons is that even in acute experiments fluid loading results in natriuresis which develops over a considerable time interval, and the major factors involved indifferent phases are probably different. In preliminary experiments we established that in rats preinfused i.v. with hypertonic saline an injection of a small fluid volume (0.5% body weight) results in an immediate major increase in sodium and water excretion. This was systematically studied in anesthetised Wistar rats prepared for clearance studies of the left kidney and measurements in its inner medulla of blood flow (MBF, laser-Doppler technique) and tissue electrical admittance (Y), an index of interstitial ion concentration. The rats were preinfused i.v. with 5% NaCl, 3 ml during 90 min. A subsequent slow injection of isotonic saline, 0.5% of body weight, increased sodium excretion (UNaV) from 2.1±0.5 to 4.5±1.1 mmol min^{-1} and urine flow (V) from 12.0±2.3 to 24.3±5.6 mL min^{-1} ($p<0.02$). The same volume of whole blood increased UNaV from 5.0±1.4 to 8.7±1.7 mmol min^{-1} and V from 22.3±5.1 to 37.4±5.9 mL min^{-1} ($p<0.01$). The glomerular filtration rate, MBF an Y did not change. In rats preinfused with 0.9% saline no natriure-

sis was observed after small volume expansion. In further studies the model of hypertonic saline loaded rat was used to examine if natriuretic prostaglandins (PG) were involved in small volume expansion (SVE) natriuresis. Indomethacin (Indo), 5 mg kg^{-1} or meclophenamate (Meclo), 7.5 mg kg^{-1}, were added to 0.9% saline injected. Paradoxically, both PG synthesis inhibitors enhanced responses to SVE. After Indo UNaV increased 3.8 fold, significantly more than after saline vehicle ($p<0.001$). At higher initial UNaV, the 3 fold increase with Meclo was significantly higher than after whole blood ($p<0.001$). MBF decreased and Y increased after both inhibitors.

We hypothesized that after PG blockade arachidonic acid metabolism in the kidney was shifted to an intact cytochrome P450 pathway. Increased production of natriuretic EETs, products of P450 epoxygenase could result in an inhibition of tubular sodium transport and natriuresis. To test this hypothesis clotrimazole (10 mg/kg body weight i.v.), a specific inhibitor of P450 epoxygenase was administered before Indo. Clotrimazole did not *per se* influence sodium excretion, however, it abolished post-indomethacin natriuresis.

We suggest that hypertonic saline loading activated the P450 pathway; PG cyclooxygenase blockade further enhanced its activity and the synthesis of epoxygenase generated compounds (EETs) which inhibited tubular sodium transport to result in pronounced natriuresis.

ROLE OF RENAL HEMODYNAMICS IN MODULATION OF THE CORTICO-MEDULLARY ELECTROLYTE GRADIENT

Supported by the State Committee for Scientific Research: grant # 4.PO5A.013.08

Research team

Janusz Sadowski, Bożena Bądzyńska, Leszek Dobrowolski, Elżbieta Kompanowska-Jezierska, Agnieszka Walkowska

First experimental evidence on interdependence of tissue ion content and blood flow in the renal medulla (conclusion)

The solute (mostly NaCl and urea) concentration in renal medullary interstitium is determined by their transport from the tubular lumen and

their dissipation by blood flowing along the *vasa recta*. The experimental data suggesting the functional role of the latter process were first provided by Thurau et al. (1962). However, due to limitations of available measuring methods, the relationship of renal medullary hypertonicity and medullary blood flow (MBF) has never been proven.

Application of tissue electrical admittance (Y) recording in the kidney *in situ* enabled continuous estimation of medullary interstitial NaCl (Sadowski, Portalska, 1983). In the years 1996-1998 we developed and applied an integrated implantable probe for simultaneous recording in rat renal medulla of Y, an index of tissue ion concentration, and MBF (laser-Doppler technique). In the *in situ* kidney of anesthetised rats these two variables as well cortical blood flow (another laser-Doppler probe on kidney surface) and aortic blood pressure were recorded simultaneously. The relationship of Y and MBF was analysed in a number of experimental series during spontaneous and drug-induced (indomethacin, glibenclamide) variations of the latter. With increases or decreases in MBF inverse changes in tissue ion concentration were clearly seen ($r = -0.77$, $p<0.01$); blood flow alterations always preceded changes in tissue admittance by 1-3 min. However, for instance, an about 50% decrease in MBF was associated with a modest 7% increase in medullary tissue Y, equivalent to a 15 mM change in tissue NaCl.

The data support experimentally the concept that the rate of medullary tissue perfusion with blood is one determinant of interstitial solute concentration. However, a short-term MBF effect on tissue ion concentration is small and could hardly be responsible for substantial rapid changes in urine concentration.

Publications

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TWENTY YEAR FOLLOW-UP AFTER THE FIRST MYOCARDIAL INFARCTION. FURTHER STUDY ON HEART FAILURE (HF) AS A COMPLICATION OF CORONARY ARTERY DISEASE

1) Incidence and significance of ventricular arrhythmias
2) The effect of creatine supplementation on the cardiovascular responses to exercise in HF patients and healthy subjects

Research team

Ewa Wójcik-Ziółkowska, Wiesława Pawłowska-Jenerowicz, Celina Romiszowska, Magdalena Płachcińska-Bijak

Serious cardiac arrhythmias and a long term outcome after the first myocardial infarction

The analysis of cardiac arrhythmias was performed in a group of 70 male patients aged 66.9±9.0 yrs, 19.3±6.6 yrs after the first myocardial infarction (MI), by 24 hour Holter ecg recording – in relation to disturbances of left ventricular contractility.

Serious arrhythmias were classified as follows:

– ventricular ectopy of the IIIrd or higher Lown class (multifocal VEBs, pairs, salvos and ventricular tachycardia – VT);

– advanced forms of supraventricular arrhythmias i.e. paroxysmal atrial fibrillation and supraventricular tachycardia.

Episodes of atrio-ventricular conduction disturbances (AV block II and III degree and pauses more than 2000 ms) were also analysed.

Rhythm disturbances were correlated with echocardiographic measurements of left ventricular function by M-mode, 2 dimensional and Doppler study.

1/ Cardiac arrhythmias are present in almost all patients in a long term follow-up (near 20 yrs) after the first MI – 97.1%.

2/ Serious ventricular arrhythmias as described above were found in 91.4% of patients.

3/ In majority of patients (64.3%) life threatening arrhythmias were noted; ventricular tachycardia occurred in about 20% of patients.

4/ Localization of the site of infarct does not identify patients with life threatening arrhythmias.

A correlation was found between the extent of left ventricular damage by echo and the intensity of ventricular arrhythmias, but this relation was not of a satisfactorily high correlation coefficient ($r<0.6$).

Monitoring of cardiac arrhythmias conducted systematically is very important for prolonging survival after MI, particularly in asymptomatic patients. In the available literature no data were found on the intensity and importance of arrhythmic complication in coronary artery disease 20 years after the first myocardial infarction.

Comparison of the autonomic nervous system reaction to postural changes and to static exercise in patients with ventricular tachycardia and those without arrhythmias

Research team

Ewa Wójcik-Ziółkowska, Celina Romiszowska

The aim of the study was to evaluate the effect of changes of the position of the body (lying, standing, sitting and standing with a weight carried in one hand) on the heart rate, blood pressure, heart rate variability (HRV) and plasma catecholamines in patients with heart failure and episodes of ventricular tachycardia (life threatening – VT) and in similar patients without arrhythmias. The study was undertaken with the presumption that the plasma catecholamine concentration would be helpful in differentiating groups and also for the discussion about mechanism of cardiac arrhythmias.

In both groups the standing position (without a carrying weight) was the strongest stimulus for an increase of circulating catecholamines (lying<standing $p<0.01$) – although the increase of noradrenaline (NA) in the VT group was smaller then that in the control group. The results are unexpected and it is necessary to complete the analysis with HRV results.

Influence of creatine supplementation on hemodynamic adaptation to exercise in patients with heart failure after MI and in healthy well trained men

Research team

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An advantageous effect of creatine supplementation on exercise capacity in healthy subjects is well known. This effect is due mainly to the enhancement of high energy phosphates in skeletal muscles. The influence of creatine supplementation on cardiac muscle has not been documented. In heart failure, the part of clinical symptoms, like fatigue and diminished exercise capacity is attributed to the metabolic changes in working skeletal muscles.

The aim of the present preliminary study was to estimate the hemodynamic reaction to treadmill exercise in patients with heart failure after MI and healthy, age matched, highly fit "old-boys cyclists".

We examined 10 men, aged 63.5 yrs – 10 to 15 yrs after myocardial infarction, with heart failure of II-III NYHA class and 7 healthy volunteers, cyclists.

The protocol of the study was as follows:

A/ first treadmill exercise test with O_2 consumption (VO_2) and cardiac output by CO_2 rebreathing method measurements; B/ echocardiographic estimation of left ventricular dimensions and function before and immediately after the exercise test; C/ 7 days' creatine supplementation (18 g per day) in oral capsules; D/ the second exercise test as described above.

It was found that the peak exercise cardiac output was diminished without an effect on VO_2 heart rate and blood pressure in almost all the patients with HF. In the healthy subjects no influence of creatine supplementation on the above parameters was noted. In the patients with HF and in 3 out of 7 healthy subjects deterioration of left ventricular contractility was found. These results encouraged us to verify the effect of commonly used creatine supplementation and to perform animal studies aiming at evaluating the effect of such a supplementation on biochemical indices of the myocardium.

Cooperating unit

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CLINICAL STUDIES ON L-ARGININE IN CORONARY HEART DISEASE AND CONGESTIVE HEAT FAILURE

Research co-ordinator
Krystyna Cedro-Ceremużyńska

Does moderate dose of L-arginine improve patients with heart failure?

In congestive heart failure, endothelial dysfunction resulting from the defect in L-arginine–NO system is manifested by diminished vasodilator response to acetylcholine and attenuated reactive and exercise hyperemia. Attempts to overcome this defect by the administration of NO substrate, L-arginine into the arterial vascular bed or systemically were successful in the majority of clinical studies. In heart failure, endothelial dysfunction may limit hyperemic response to exercise and thereby lower exercise capacity. Therefore, the question whether L-arginine can improve exercise tolerance in heart failure, seems of particular importance. Two studies published so far on this subject reported improvement (Rector et al., *Circulation* 1996, 93, 2135) or null effect (Chin-Dusting et al., *JACC* 1996, 27, 1207) of L-arginine on functional capacity and endothelial function. In view of these conflicting results, we designed a pilot investigation to confirm or refute the concept that moderate doses of L-arginine favourably influence functional and biochemical parameters in chronic heart failure. The patients enrolled into this randomized double-blind, placebo-controlled study, suffering from chronic heart failure had to fulfill the following entry criteria: functional class NYHA II-IV, ejection fraction <40%, stable clinical condition, unchanged medications for at least one week before the enrollment, no overt atherosclerotic peripheral disease. Pharmacological treatment was unchanged throughout the study. Patients were randomly assigned to L-arginine (3.0 g

3 times daily) or placebo for 1 week. Treatment was crossed over after 1 week of the wash-out period. Treadmill exercise test (Naughton protocol) and blood sampling were performed at the begining of each week and at the end of the whole study period. Results obtained so far in 12 patients have shown that the dose of L-arginine was well tolerated and blood levels of urea, creatinine and electrolytes were unaffected. However, neither total exercise time(s) nor maximal work load (METS) were significantly influenced by L-arginine supplementation. The markers of free radical activity (plasma lipid peroxides, oxygen free radical production by leukocytes) and free radical defense mechanisms (serum thiols) as well as platelet aggregation remained unchanged. Before final conclusions are drawn from this investigation, we aim to enlarge the study group and to evaluate whether the patients in whom chronic heart failure is combined with diabetes and/or dyslipidemia differ in their response to L-arginine.

L-arginine in coronary heart disease

A clinical study on the effect of L-arginine on the course and outcome of myocardial infarction (MI) is at the stage of organisation. This will be a prospective, multicenter, randomized, double blind, placebo controlled study designed to elucidate whether L-arginine or antioxidant vitamins C and E administered orally for 30 days from the onset of MI influence the incidence of important complications, mortality and prognosis during 30 days and 6 month observation periods.

Publication

Budaj A, Herbaczyńska-Cedro K., Kokot F., Ceremużyński L.: Effect of early captopril treatment on blood adrenaline levels in acute myocardial infarction. The substudy of ISIS-4. *Am J Cardiol* 1998, 81, 335-339.

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SKIN IMMUNE CELLS AND CYTOKINES IN INFLAMMATION

Research team

Waldemar L.Olszewski, Hanna Gałkowska

The cellular and humoral composition of tissue fluid and lymph reflects tissue reaction to local antigenic response. This has been proved in our original model of chronic collection of human and animal lymph draining from the sites of antigen deposition. The intensity of local response can be quantitated basing upon the concentration of inflammatory proteins, cytokines and numbers of activated immune cells. We observed in patients with rheumatoid arthritis high levels of proinflammatory cytokines as IL1, IL6, TNF alpha, antiinflammatory cytokines as IL10 and IL1Ra in lymph draining the inflamed joints. The serum cytokine levels remained normal or were slightly elevated. High levels of cytokines in tissue fluid suggest their local production by infiltrating and activated resident cells.

The acute phase proteins produced elsewhere are represented in tissue fluid at concentrations reaching 25% that of plasma. This indicates that the inflammatory proteins are synthetized locally but delivered from circulation as capillary filtrate.

The method of tissue fluid/lymph collection gives an excellent insight into local events in tissues.

Cooperating units

Department of Cardiovascular Biochemistry, St. Bartholomew's Medical College, London, England
Institute of Rheumatology, Warsaw, Poland

TOLERANCE TO ALLOGENEIC TRANSPLANTS

Research team

Waldemar L.Olszewski, Barbara Łukomska, Marek Durlik

Transplantation of allogeneic bone marrow is burdened by a high degree of elimination of infused hematopoietic cells. This phenomenon is called hybrid resistance. Although it is mostly expressed in rodents, a human equivalent of this phenomenon can also be observed. We showed that transplantation of bone marrow in rat hind-limb as a fully vascularized tissue with hematopoietic cells in physiological spatial relationship with stromal cells enables full repopulation of the recipient with as few as several thousand of donor cells released from the graft. In order to prevent the nonspecific elimination of some of the transplanted cell by recipient NK cells, the anti-AGM1 antiserum was administered to recipients with satisfactory results.

Interestingly, co-transplantation of vascularized bone marrow and skin from the same donor significantly prolonged the survival time of the latter. Microchimerism could be observed in recipient peripheral lymphoid tissues. Donor-specific cells were detected by both tissue specimen staining and flow cytometry. The eventual role of microchimerism in development of tolerance to donor transplantation antigens remains to be further substantiated.

TRANSPLANTATION OF CELLS AND LOCAL ALLOGENEIC REACTION

Research co-ordinator

Waldemar L.Olszewski

Hepatocyte transplantation.

One of the obstacles in effective hepatocyte transplantation has been rapid destruction of transplanted cells. We showed that the mediator of elimination of subcutaneously, intraperitoneally and intravenously transplanted hepatocytes are granulocytes and macrophages. Depletion of these populations resulted in increased recovery of transplanted cells. Blocking of hepatocyte surface antigens was followed by higher recovery rate. Also,

an *in vitro* test was developed based on the phenomenon of cluster formation between hepatocytes and attacking granulocytes and mononuclear cells. Preliminary observations point to high rate of cluster formation. This process was found to be time, and Ca++ and Mg++ dependent.

The effect of cyclosporin A on lymphocyte migration.

Lymphocytes pretreated with cyclosporin A reveal decreased migrational properties to the allografts. We found that CsA suppresses expression of L-selectin on lymphocytes. This effect may have influence on *in vivo* lymphocyte traffic.

SECONDARY LYMPHEDEMA IN VENOUS STASIS, AFTER TRAUMA AND SKIN INFECTIONS - THE ROLE OF BACTERIAL FACTOR

Supported by the State Committee for Scientific Research: grant # 4.P05C.079.13

Research co-ordinator

Waldemar L.Olszewski

Lymphoscintigraphic and bacteriological studies were carried out in a second group of patients with lymphatic obstruction. No correlation between the intensity of scintigraphic changes and the degree of colonization of leg tissue and lymph could be found. We isolated microorganisms from 20% of inguinal lymph nodes of patients with lymph stasis. These were *Staphylococcus epidermidis*, *Acinetobacter* and coagulase-negative *Staphylococci*. No isolates could be found in control healthy subjects. Comparison in some cases of two strains of *Staphylococcus*, one from the toe skin surface and the other from the inguinal lymph node revealed identical DNA pattern. This finding indicates that microorganisms can readily penetrate skin and locate in deep tissues. In lymph stasis, the microorganisms remain in tissues deprived of tissue fluid and lymph flow, and occasionally proliferate evoking major inflammatory reaction.

Cooperating units

Indian National Science Academy, New Delhi, India
The Norwegian Radium Hospital, Oslo, Norway

THE MECHANISM OF FAST HEMATOPOIETIC RECONSTITUTION IN RATS AFTER VASCULARIZED BONE MARROW TRANSPLANTATION

Supported by the State Committee for Scientific Research: grant # 4.PO5A.049.15

Research team

Barbara Łukomska, Marek Durlik, Bożenna Interewicz, Sława Janczewska, Waldemar L.Olszewski

Lymphocyte recovery after high-dose chemotherapy and intravenous bone marrow transplantation (BMT) has been slow and resulting in persistent immune deficiency. We developed a model in which complete lymphopoietic reconstitution could be obtained in lethally irradiated rats by transplantation of vascularized bone marrow (VBMTx) in orthotopic hindlimb graft. The study was devoted to the problem of the role of transplanted donor origin and remnant recipient hematopoietic cells in replenishing host lymphocyte pool. Hind limbs were transplanted orthotopically into irradiated (8Gy) syngeneic sex-mismatched recipients. In another group 8x107 BMC were injected i.v. (BMCTx) in the same sex-mismatched combination. PCR analysis of material collected 10 days after grafting was performed using specific primers for rat Y chromosome (sex-determining region Y-Sry). The density (amount of donor DNA) of the band corresponding to Y-Sry in peripheral blood mononuclear cells and mesenteric node lymphocytes of VBMTx female recipients was higher than of BMCTx rats (OD 0.48 *vs* 0.22 and 0.23 *vs* 0.01, respectively). Interestingly, in the female to male combination a high amount of host DNA was detected in bone marrow and blood cells as well as node lymphocytes of VBMTx rats but not in similar tissues of BMCTx recipients. These data indicate that VBMTx containing stromal cells together with BM hematopoietic cells provides necessary signals for efficient proliferation and maturation of donor cells in lethally irradiated rats and concomitantly stimulates host precursors to generate new lymphocytes. Lymphocyte recovery after BMCTx was evidently less expressed and was mainly the effect of proliferation of donor but not recipient hematopoietic cells.

IDENTIFICATION OF GROWTH FACTORS IN LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN PATIENTS WITH LIVER TUMORS

Supported by the State Committee for Scientific Research: grant # 4.PO5C.045.14

Research team

Barbara Łukomska, Joanna Dłużniewska

The adult liver retains the capacity to restore its mass in response to partial hepatectomy. Resection of liver tissue initiates the release of local growth factors that results in proliferation of all hepatic cellular elements. Whether these growth factors are involved in the post-resection regeneration of liver in patients with benign and metastatic tumors remains unknown. Aim of the study was to investigate whether there exists a relationship between the expression of local growth factors in liver cells and the rate of liver tissue regeneration after partial hepatectomy for liver tumors. Patients undergoing partial hepatectomy for benign tumors and colon adenocarcinoma metastases were studied. Liver volume was measured in spiral CT before and 30 days after surgery. Immunohistochemical examination for the presence of PCNA, Ki67, HGF, c-Met, TGF alpha, and EGF-R was performed on a) sections of resected fragments of liver tissue remote from the tumor, b) liver biopsy specimens taken before closing the abdomen, and c) fine needle aspiration biopsy material 7 days after liver resection. Evident regeneration of liver tissue was seen after resection, with higher rate in benign than malignant tumor group. Low expression of HGF was detected in nonparenchymal cells of excised liver tissue. Seven days after liver resection HGF was overexpressed in perisinusoidal, Kupffer and sinusoidal endothelial cells. cMET was expressed in hepatocytes of resected liver tissue. Elevated expression of cMET was observed in the regenerating liver. TGF alpha was not found in the resected liver tissue but a low expression of this cytokine was detected in specimens 7 days after hepatectomy. Staining for proliferating cell nuclear antigen (PCNA) and Ki67 was negative in normal liver tissue. A significant rise of the expression of both these antigens was detected in regenerating liver. No differences in the expression of growth factors were noticed in the regenerating liver tissue taken from livers with benign or metastatic tumors despite of different rate of liver regeneration in patients with these two types of tumors.

Cooperating unit
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PATHOMECHANISM OF PROTRACTED WOUND HEALING DISORDERS IN THE PRODUCTION OF GROWTH FACTORS REGULATING FIBROBLAST AND KERATINOCYTE PROLIFERATION PROPOSED THERAPY

Supported by the State Committee for Scientific Research: grant # 4.PO5C.030.10

Research team
Hanna Gałkowska, Waldemar L. Olszewski

In chronic wounds the disturbances in formation of granulation tissue and deficient reepithelialization occur. Understanding of interaction between factors responsible for reepithelialization will allow to correct delayed wound healing. The present study was carried out in patients with leg varicous ulcer and arterial ischemia undergoing arterial surgery. The presence of fibroblasts, macrophages and granulocytes in wound and their ability to phagocytize were analyzed. The neovascularization process (expression of factor VIII, CD31) and the presence of adhesion molecules responsible for leukocyte extravasation (CD62E, VCAM1) were estimated. Also expression of extracellular matrix proteins – laminin, fibronectin, tenascin, vimentin, collagen IV and VII were studied. The presence of metalloproteinases and their inhibitors responsible for remodelling of extracellular matrix and granulation tissue resorption due to apoptosis (expression of p53 protein, Bcl-2, Bax, CCP-3, TUNEL) was analyzed. Proliferation of keratinocytes (PCNA, CK16), their migration (CD51) and differentiation (CK17) and ability to produce angiogenic factor – VEGF were investigated. The neovascularization process and proliferation as well as differentiation of Langerhans cells were found slow. High degree of apoptosis could be seen in ulcer bed and adjacent areas. Adhesion molecules were weakly expressed in these areas.

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PATHOMECHANISM OF POSTTRAUMATIC EDEMA IN THE LOWER EXTREMITIES

Supported by the State Committee for Scientific Research: grant # 4 P05C 037 10

Research team

Grzegorz Szczęsny, Waldemar L. Olszewski

The mechanism and pathophysiology of lower leg edema after mechanical trauma were investigated both in clinical practice and in experiment.

1. The role of lymphatic drainage in the pathomechanism of persistent edema was investigated in 21 patients sufferring from mechanical injury (fracture, dislocation, torsion or contusion) with edema of 3 months duration. In each patient increase in limb circumference and local skin temperature of injured legs were observed. Lymphoscintigrammes showed pictures of dilated lymphatic trunks and enlarged inguinal lymph nodes. Interestingly, there was no interruption of lymphatic pathways. USG-Doppler investigation of limb veins revealed presence of thrombotic deep venous system in five patients (24%). The obtained data point to the presence of inflammatory process at the site of injury with high rate of lymph production.

2. Investigations on dogs with subcutaneous injection of fluorescein-labelled blood cells showed, that the speed of elimination of blood cellular elements from soft tissues is relatively slow amounting to 0.75±0.8% of the injected dose for erythrocytes and 1.42±2.2 for leukocytes. Platelets were not drained through lymphatics. In other experiments 10^7/0.5 ml of skin saprophyte bacteria (*Staphylococcus simultans*, *Staphylococcus capitis* and *Micrococcus species*) were injected daily over 6 weeks in hind paws. Direct lymphographies performed two weeks after the last injection showed increased numbers and dilatation of lymphatic vessels as well as enlargement of lymph nodes. Results of experimental studies suggest that blood extravasation is not responsible for the local "inflammatory" changes in soft tissues. The bacterial factor may play a role in local lymph production and secondary changes in lymph nodes.

ACCUMULATION OF LYMPHOCYTES IN LIVER SINUSOIDS AND THEIR ROLE IN LOCAL REACTION AGAINST TUMOR CELLS

Supported by the State Committee for Scientific Research: grant # 4.PO5A.047.12

Research team

Sergiusz Durowicz, Joanna Dłużniewska, Dorota Laszuk, Barbara Łukomska, Waldemar L. Olszewski

Immune function of liver is associated with a specific lymphocyte population marginating in liver sinusoids. This population is responsible for destruction of malignant cells reaching liver with the splanchnic blood. In our previous studies we found that in a normal liver the liver-associated lymphocytes express high cytotoxic activity against tumor cells. Moreover, the presence of liver tumor metastases (raised by intraportal inoculation of CC531 colon adenocarcinoma cells) is connected with decreased cytotoxic activity of liver sinusoidal lymphocytes, whereas the cytotoxicity of peripheral blood lymphocytes remains unchanged. The aim of the study was to characterise the adhesion molecules regulating attachment of lymphocytes in sinusoids of tumor bearing liver.

Immunohistochemical analysis of liver associated lymphocytes from tumor bearing and healthy liver showed no difference in the percentage of cells expressing CD54 (ICAM-1), Cd11a (LFA-1 alpha), CD11b (MAC-1 alpha), CD18 (LFA-1 alpha), CD49d (VLA-4) between those two groups. It seems that the observed low cytotoxic activity of liver associated lymphocytes in tumor bearing liver is not due to the weak expression of adhesion molecules.

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STRUCTURE-ACTIVITY STUDIES OF OPIOID PEPTIDES

This year activities were concentrated on (i) structure-activity studies of biphalin and (ii) search for new opioid peptide analogues as a potential analgesic drugs. The tetrapeptide dimer, biphalin, originally synthesized in this laboratory possesses unique properties comparing to many other opioid peptide analogues studied since. Biphalin is reported to have high affinity for all three opioid receptor types (μ, δ and κ) in radioligand binding studies and is an extremely potent analgesic in *in vitro* tests. In collaboration with Professor Hruby group (University of Arizona, Tucson) we were continuing structure-activity studies of biphalin. We were able to establish that only a part of the molecule is responsible for its high analgesic activity. In collaboration with Professor Specter group (University of Florida) we described reverse transcriptase inhibitory activity of biphalin. This discovery may influence future applications of biphalin in clinical use. In collaboration with Professors Carr and Kream (New England Medical Centre, Boston), we developed a new group of analgesics, which are hybrides of opioid peptides with other neuropeptides. Our group also participated in development of new opioid peptidomimetics by Professor Zabrocki group (Technological University, Łódź).

PEPTIDES WITH AMPHIPHILIC AMINO ACIDS

Supported by the State Committee for Scientific Research: grant # 3T09A07612

Hydrophobic and aromatic interactions are most critical for membrane peptide receptor-ligand complex stability. We have hypothesised that proper location of the hydrophilic counterparts to lipophylic and/or aromatic residues may stabilise the complex with the receptor pocket. Presently we have studied the consequences of introduction of α-hydroxymethyl group into the α-position of phenylalanine or tyrosine residues of enkephalin or deltorphin analogues. We observed that the significance of such a modification was strongly dependent on the position of the primary amino acid in the peptide chain. The study was accomplished with collaboration with Dr. Olma (Technological University, Łódź), and Professor Tourwe (Vrije Universiteit, Brussel).

OTHER SCIENTIFIC ACTIVITIES

Our laboratory has long term scientific collaboration with the Industrial Chemistry Research Institute (Warsaw). This year results of this collaboration were: (i) development of a new method of enzymatic resolution of amino acid derivatives and a study on the possible application of spinal cord hydrolizates for the oral tolerance development in multiple sclerosis treatment.

In collaboration with Professor Jerzmanowski group (Warsaw University) we have studied structure determinants of short peptides interacting with AT-rich DNA.

In collaboration with Professor Szczepańska-Sadowska group (Warsaw Medical School) we accomplished study of effect of nitric oxide donor, SNAP on blood pressure in SH-rats.

Cooperating units

Massachusetts General Hospital, Harvard Medical School, Boston, USA (S.K. Szyfelbein)
University of Arizona, Tucson, USA (V. Hruby)
University of Florida, USA (S. Specter)
New England Medical Centre, Boston, USA (D. Carr, R. Kream)

Vrije Universiteit, Brussel, Belgium (D. Tourwe)
Technological University, Łódź, Poland (J. Zabrocki, A. Olma)
Industrial Chemistry Research Institute, Warsaw, Poland
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Publications

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AUTOANTIBODIES REACTIVE WITH EXTRACELLULAR MATRIX (ECM) PROTEINS IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY (TAO)

Supported by the State Committee for Scientific Research: grant # 4.PO5A.138.14

Research team

Tomasz Bednarczuk, Zbigniew Bartoszewicz, Jacek Kiljański, Janusz Nauman

Thyroid-associated ophthalmopathy (TAO) is now accepted as an autoimmune inflammatory disorder of extraocular muscles and periorbital connective tissue. Histopathologic examinations of tissues obtained at autopsy and from decompression procedures indicate that the inflammation takes place in the endomysial, perimysial and periorbital connective tissue.

Autoantibodies recognizing uncharacterized connective tissue/perimysial components have been demonstrated in patients with TAO. Because of the possibility that such antibodies may play a role in the autoimmune inflammation, we studied the humoral immune response against specific extracellular matrix (ECM) proteins, namely: the pericellular connective tissue components fibronectin (FN), collagen types I, III, V (CI, CIII, CV) and the basement membrane proteins collagen type IV (CIV) and laminin (LM) using specific immunoassays.

Sera for the studies were obtained from 66 patients with TAO and, for comparison, from 63 patients with Graves disease (GD) without clinical eye involvement; 45 patients with Hashimoto's thyroiditis (HT); 37 patients with non-autoimmune thyroid disease (nAITD); and from 63 normal subjects.

The study suggests that a variety of ECM proteins (CI, CV, LM) may be secondary autoantigens that are recognized by antibodies in TAO. While

these antibodies appear to react with epitopes expressed on both native and denatured proteins, and may therefore have the potential to bind to ECM *in vivo*, their pathogenic role in TAO remains unclear. However, antibodies reactive with connective tissue proteins may contribute to tissue damage, for instance by complement fixation.

Cooperating units

Department of Endocrinology, Warsaw University School of Medicine, Poland.

Department of Endocrinology, Kurume University Medical School, Kurume, Japan.

EXPRESSION AND FUNCTION OF THYROID HORMONE RECEPTORS (TR) AND 9-CIS RETINOID ACID RECEPTORS (RXR) IN THYROID CANCERS (DEPENDENT ON IRRADIATION?)

Supported by the State Committee for Scientific Research: grant # 4.P05B.041.15

Research team

Monika Puzianowska-Kuźnicka, Janusz Nauman

Studies are in the preliminary stage. Sofar, fragments of thyroid tissues, were excised during thyreoidectomy performed due to: a) struma nodosa (benign changes, first control group consisting of 10 patients); b) adenoma (benign changes, second control group, also 10 patients); c) thyroid cancer (7 patients).

Since the amount of excised tissue (especially cancer and adenoma tissue) was very limited, total RNA from all control tissues has been isolated using Mini-RNA kit (Qiagen) designed for this purpose. The RNA yield was 5 - 20 mg. We have started cloning the full length translated regions of TRa1 and TRb1 using the RT-PCR method. Sofar, all the TRa1 and TRb1 from group a) has been cloned into pGEM-T vector (Promega).

Cooperating unit

Medical Centre of Postgraduate Education, Warsaw, Poland (M. Ambroziak).

THYROID HORMONE RECEPTORS (TR) AND 9-CIS RETINOID ACID RECEPTORS (RXT) IN HUMAN CLEAR CELL KIDNEY CANCER

Research co-ordinator

Monika Puzianowska-Kuźnicka

The expression of TR genes on the mRNA level was studies in fragments of human clear cell kidney cancers and the kidney tissue surrounding the tumor, obtained from 20 patients. The cancers have been divided into three groups: a) well differentiated, b) showing intermediate level of differentiation, and c) poorly differentiated cancers. In addition, fragments of kidney tissue were obtained during nephrectomies performed due to reasons other than cancer (urinary calculi, ectopic kidney, etc.). Total RNA has been isolated using TRI-zol reagent (Gibco) and northern blots were made. Incubation with TRa or TRb probes revealed the amount of specific mRNA in cancer tissues to decrease with their differentiation level.

Cooperating units

Department of Biochemistry, Medical Center of Postgraduate Education, Warsaw, Poland (A. Nauman)

National Cancer Institute, NIH, Bethesda, USA (Sheue-Yann Cheng)

Publications

Kuźnicki J., Puzianowska-Kuźnicka M.: Calcium ions and apoptosis. *Postępy Biologii Komórki* 1998, 25 (Suppl 11), 29-42 (in Polish).

Nauman A., Puzianowska-Kuźnicka M., Pietrzykowski A., Grzesiuk W., Luczak J., Nauman J.: Iodothyronine-5'-deiodinase in atrium and ventricle of rat heart. *Endokrynol Pol* 1998, 49, 261-270.

PROMOTIONS

HABILITATION THESIS

Krystyna Budzińska
Control of respiratory activity

Józef Langfort
Effect of low-carbohydrate diet on exercise tolerance, metabolic and hormonal responses to exercise of various characteristics (habilitation at Medical University School in Białystok)

Teresa Zalewska
Kinases (PKC and CaMKII) and proteases as sensors of cerebral ischemia induced calcium signal

DOCTOR'S THESIS

Tomasz Bednarczuk
Interaction of T-cells with extracellular matrix proteins in patients with thyroid-associated ophthalmopathy

Leszek Czerwosz
A new multifunctional software for recording of biological signals. Application for electronystagmography

Małgorzata Dorobek
Analysis of clinical and genetical heterogeneity of limb-girdle dystrophies

Alicja Kodrzycka
Comparison of the autonomic nervous system activity in sedentary men and in elite swimmers (defended in Academy of Physical Education, Warsaw)

Elżbieta Kowalska-Olędzka
Clinical symptoms and immunologic and immunogenetic markers in patients with idiopathic inflammatory myopathies

Andrzej Namysłowski
Mechanism of immunological enhancement in allografts – distribution of alloantigen and alloserum in the recipient

ORGANIZATION OF SYMPOSIA AND CONFERENCES

"Neurological syndroms in vasculitis", Warsaw, February 20, 1998.

Conference of Commision of Neuromuscular Pathology – "Inflammatory myopathies", Warsaw, April 16, 1998.

"Magnetic Resonance Spectroscopy *in vivo*", Meeting of the Comission of Radiology of the Committee of Pathophysiology, Polish Academy of Sciences, Warsaw, June 5, 1998.
The symposium was organized by the Laboratory of Experimental Pharmacology of the Medical Research Centre. Number of participants – 40. The meeting was devoted to the practical (technological and methodological) aspects of the proton, as well as phosphorous magnetic resonance of spectroscopy of human brain and muscles *in vivo*. Five invited lectures were presented, concerning achievements and limitations of *in vivo* resonance spectroscopy, and current state of this techniques in leading domestic centres. The conference was an important step towards standardization of magnetic resonance spectroscopy in Poland.

Symposium: „Progress in diagnostics and treatment of obliterative atherosclerosis", Warsaw, September 30, 1998.

Symposium: „Progress in transplantation of liver", Warsaw, October 28, 1998.

Symposium: „Disorders of immunity in patients after injuries, with neoplasms, infections and after transplantations", Warsaw, November 25, 1998.

IV Neurochemical Conference: „Molecular basis of pathology and therapy of neurological diseases", Warsaw, December 11, 1998.

GUEST LECTURES

Prof. Arne Schousboe (Royal Danish School of Pharmacy, Copenhagen): „Role of calcium and receptor desensitization in AMPA and kainate mediated neurotoxocity" (September 1998).

Dr Sheue-Yann Cheng (National Cancer Institute, NIH, Bethesda, USA): "Thyroid hormone receptors in hepatocellular cancer", May 1998.